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With the Collaboration of

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ABSTRACT

This bulletin covers a comprehensive study of blood changes occurring in hemorrhagic anemia induced in dogs maintained on a synthetic diet. A comparison is drawn between the blood picture observed in this experimental anemia and that noted in idiopathic hypochromic anemia.

Many data, consisting of physical and chemical measurements on the blood of normal dogs, are presented. These data, used in selecting normal animals for the experiments, represent the goals of achievement in evaluating nutritional programs designed to restore lost blood.

The following factors return to normal relatively rapidly after bleeding: plasma volume, total calcium and phosphorus in the circulating plasma, serum albumin, and fibrinogen.

Factors that return to normal slowly or remain persistently below normal modify the blood picture and become the measurements used in assessing any corrective nutritional regimé. These include individual red cell volume and diameter, total blood volume, total cell volume, cell volume per cent, hemoglobin, serum globulin, and protein nitrogen of whole blood.

Constituents for which no marked trends were observed are leucocytes, nonprotein nitrogen, urea, creatinine, and amino acid nitrogen.

Statistically established interrelationships among certain of the variables are discussed.

The implications of the similarities between idiopathic hypochromic anemia and hemorrhagic anemia are considered. The anemia induced by hemorrhages, uncomplicated by nutritional deficiencies, pregnancy, lactation, menstruation, or infections, is of the hypochromic microcytic type.

The Blood Picture in Hemorrhagic Anemia

I. INTRODUCTION

THE TERM ANEMIA covers a number of abnormal conditions of the blood in which changes are found in the hemoglobin content, or the number, size, and composition of erythrocytes.

The causes of anemia are as varied as the blood pictures. In nutritional anemia there is a deficiency of substances essential to the synthesis of hemoglobin, such as iron or copper. In pernicious anemia there is a lack of compounds needed for building the stroma of the red blood cells. In hemorrhagic anemia there are abnormalities which arise from blood losses. In the latter an individual's condition may be seriously affected by the amount of blood lost in a single hemorrhage, by the total amount of blood lost in a series of small hemorrhages, or through continuous slow bleeding.

Hemorrhages occur in the alimentary or genito-urinary tracts, in accidents, operations, childbirth, or menstruation. In lesions in the alimentary tract or in the genito-urinary tract, blood is commonly mixed with secretions or other materials which make accurate measurement difficult. Ordinarily, accidents take place under conditions which preclude measurement of the blood lost. Data compiled in table 1 illustrate blood losses occurring in operations, childbirth, and menstruation, as cited in the literature.

The seriousness of the hemorrhages recorded in table 1 may be appreciated, in part, by comparing the means and ranges of blood losses with available data on total blood volumes in men and women. Rowntree, Brown, and Roth (1929) reported a mean blood volume of 6,040 cc. (S.D. = 758 cc.) for 49 healthy men, and 5,076 cc. (S.D. = 480 cc.) for 25 normal women. The loss of a few cubic centimeters of blood will, therefore, constitute a very small percentage of the total volume, whereas a liter will be equivalent to a fifth or a sixth of an adult's total blood volume.

The significance of the hemorrhages shown in table 1 depends upon the rate at which bleeding occurs, upon the relative amount or percentage of total blood volume involved, upon the ease with which an individual replaces the constituents of the blood, and upon the interference, caused by hemorrhage, with normal physiological functions of the body. A large hemorrhage may be serious because of the relatively great quantity of

blood lost in a brief period and an individual's inability to adjust himself to the sudden change. Small repeated losses may become grave because of their cumulative effect and failure of the organism to recover from one hemorrhagic loss before another hemorrhage occurs. Thus Wright

Table 1. Sizes of Hemorrhages Reported in the Literature

Nature of hemorrhage	No. of cases	Mean loss in cc.	Range of losses in cc.	Investigator
Operative				
Miscellaneous	35	172	4 to 816	Gatch & Little (1924)
Miscellaneous	18	195	8 to 1,272	Coller & Maddock (1932).
Miscellaneous	74	459*	10 to 1,900*	Buddington & Taylor (1938)
Prostatic resections	55	479		Pilcher & Sheard (1937)
Prostatic resections, with improved technique	55	291		Pilcher & Sheard (1937)
Nose, throat, ear	924	165	0 to 887	McKenty (1937)
Postpartum	1,870	271		Pastore (1937)†
Menstrual	50	37	9 to 207	Barer, Fowler, and Baldridge (1934-35)
Menstrual	100	51	7 to 179	Barer & Fowler (1936)
Menstrual	4	33	24 to 52	Leverton & Roberts (1937)

* Estimated from graphs.

† 133 patients, loss of 600 cc. or more. Maximum of 1,650 cc. in 2,370 cases.

(1933), Barer, Fowler, and Baldridge (1934-35), and Barer and Fowler (1936) postulated that in some women who suffer from microcytic or hypochromic anemia of undetermined origin, the iron or hemoglobin lost during menstruation is not replaced entirely before subsequent menstrual periods, with the result that a persistent anemia develops. Hypochromic anemia is found infrequently in men, and Witts (1931) regards its occurrence as evidence of blood loss.

The primary objective of this investigation was to secure a comprehensive picture of blood changes occurring in hemorrhagic anemia. Using normal adult dogs as experimental animals, bleedings were induced to simulate in size and number hemorrhages that occur in human subjects under such conditions as those following accidents, childbirth, excessive menstrual losses, and the like.

A further objective was to point out the factors constituting the most accurate gauge of the status or condition of an individual experiencing loss of blood, as well as those which most accurately measure progress made toward recovery. Those which return to normal slowly, or remain persistently below normal, become the problems for which solutions are most urgently needed. Any corrective regime to be adjudged wholly successful must accomplish a complete restoration of all of these factors.

The present investigation dealt with changes that occurred in the blood picture of 19 male and female dogs, observed for varying lengths of time following the removal of significant amounts of blood. This

bulletin presents a comprehensive description of the changes resulting from severe hemorrhages and the progress of recovery in the animals, maintained on what is believed to be an adequate synthetic diet for adult dogs.

Those factors in the blood picture which changed after bleeding were carefully observed in order to ascertain which of them returned to normal rapidly and apparently with ease, and which of them did not return to normal at all or whose return was greatly delayed.

The authors accumulated considerable data comprising physical and chemical measurements on the blood of 50 normal dogs. These were of value for making comparisons between prehemorrhagic and post-hemorrhagic blood pictures and for selecting animals suitable for these experiments. In evaluating any nutritional program designed to restore lost blood, the normal blood picture becomes the goal of achievement.

Since the inception of this research, extensive literature relating to idiopathic hypochromic anemia has appeared. A comparison will be drawn between the blood picture observed in this type of anemia and that noted in experimental hemorrhagic anemia, and the implications of the similarities noted will be discussed.

II. METHODS

SELECTION AND CARE OF EXPERIMENTAL ANIMALS

NORMAL ADULT DOGS were considered most acceptable for the purposes of these experiments, since it has been found that they can be maintained satisfactorily for many months on a synthetic diet. Various foods can be substituted for a part of the synthetic ration and can be tested. The blood volume of dogs can be determined with relative ease, and aliquots of the previously measured blood volume can be removed to induce hemorrhages of desired severity. Furthermore, working with dogs, it is possible to remove sufficiently large samples of blood to permit numerous simultaneous physical and chemical measurements.

Although hemorrhagic anemia occurs frequently in human subjects as a result of blood losses, often the size of a hemorrhage cannot readily be determined. Sometimes investigators find complicating conditions of pathological or physiological nature. Moreover, human subjects are not available for experimental procedures in which measured amounts of blood must be withdrawn, the diet restricted and controlled over considerable periods of time, and significant amounts of blood removed at frequent intervals for determining blood volumes and securing various other physical and chemical measurements.

Every effort was made to select adult dogs in good physical condition and of sizes suitable for the hemorrhages and blood sampling an-

ticipated. During a preliminary laboratory period, the dogs' reaction to the synthetic diet and their fitness for blood sampling were carefully observed. The animals were kept in individual cages, in a well-ventilated, sunny laboratory. Care was taken to maintain a comfortable temperature, and to keep the dogs, the laboratory, and all equipment scrupulously clean.

SYNTHETIC DIET

The synthetic diet used was a modification of that proposed by Karr (1920) and consisted of:

	Parts by weight
Casein	6.30
Sucrose	4.50
Cod-liver oil	0.60
Lard	3.30
Bone ash	0.25
Salt mixture	0.20
Dried yeast	0.50

The formula for the salt mixture was as follows:

	Parts by weight
Sodium chloride	10.0
Calcium lactate	4.0
Magnesium citrate	4.0
Ferric citrate	1.0
Copper sulfate (crystalline)	0.2
Potassium chloride	1.0

The dogs were given sufficient food to maintain a weight equilibrium. The adequacy of this ration was shown by the fact that three dogs, numbers 57, 58, and 59 (table 2), kept on the diet exclusively for 20 months except for those periods when test foods were added, remained in excellent physical condition throughout the experiment. Furthermore, four dogs, numbers 36, 37, 38, and 39 given the same diet exclusively for six to eight months, were also adjudged perfectly healthy at the end of the investigation. None of the commonly accepted evidences of vitamin deficiency were noted at any time.

EXPERIMENTAL PROCEDURE

Four groups of animals were used for this investigation. In all cases reported, anemia was induced through the removal, by venipuncture, of a measured amount of blood from the jugular vein.

The first group comprised four male dogs, numbers 44, 45, 46, and 47, maintained at a reasonably constant weight on the synthetic diet already described. For convenient reference, this series of experiments will be designated as the "short-term study." That each dog might

serve as his own control, normal levels for all the factors studied were set by at least four determinations before each unit hemorrhage had removed approximately 20 per cent of the previously established total blood volume. Samples were taken for subsequent measurement, first, within 30 minutes after the start of bleeding, and thereafter on each of the five or six days immediately following. To portray prebleeding and postbleeding levels adequately, it was necessary to remove from each animal daily 65 to 70 cc., representing approximately five per cent of the total blood volume. While these are in fact appreciable blood losses, they are nevertheless comparable to similar losses often experienced before or after severe hemorrhages. In adult human subjects a unit hemorrhage of this magnitude would correspond to the loss of one quart of blood in a single hemorrhage, followed by smaller losses during the week following. Three of the dogs in this group were subjected to two large bleedings; the fourth was bled four times at monthly intervals. After hemorrhage, each animal was allowed a recovery period of not less than four weeks, and during the first two weeks of recuperation 100 grams of beef liver daily replaced a part of the synthetic ration.

The second group consisted of nine animals, five females, numbers 20, 21, 22, 19, and 32, and four males, numbers 17, 18, 30, and 31. The dogs were maintained on the same synthetic diet as that used for the short-term study. Normal levels were established by two or more determinations, following which the animals were rendered anemic by two large bleedings spaced two or three days apart, in each of which one fourth of the predetermined total blood volume was removed. These two large bleedings represent as severe hemorrhages as human subjects can experience without fatality. Weekly determinations were made for a long period, 12 weeks in most cases, during which the progress of recovery was carefully observed. For convenient reference, this second series of experiments will be designated as the "long-term study".

In a third study, three additional male dogs, numbers 36, 37, and 38, were maintained on the same synthetic ration for a period of approximately six months. Normal levels were established as before, after which these animals were subjected to repeated bleedings (four or five). Numerous data were accumulated covering these animals which will be reported in a later publication. In this bulletin, however, only that portion of the data acquired which dealt with changes in cell size and total and differential white cell counts will be discussed.

A fourth group of dogs, consisting of three males, numbers 54, 55, and 56, were subjected to repeated bleedings (three or four), following the establishment of normal levels. These dogs were also maintained on the synthetic ration used for the other studies and were observed for a period of three to three and one-half months. Again considerable data were accumulated, but reference in this bulletin is confined to that portion pertaining to plasma protein fractions.

These four studies are thought to be representative of various combinations of large and small hemorrhages which occur in human subjects, with respect to quantities of blood lost and the rate of bleeding. It is anticipated that the data obtained will give a complete picture of the changes occurring in the blood following hemorrhage and will throw light on some of the difficulties inherent in complete recovery.

PHYSICAL MEASUREMENTS ON BLOOD

Blood volume was measured, using Hooper, Smith, Belt, and Whipple's (1920) modification of Keith, Rowntree, and Geraghty's (1915) dye method. To make fair comparisons among dogs of different sizes, both blood volume and red-cell volume were expressed in terms of cubic centimeters per kilogram of body weight. Cell volume per cent was also included in the data.

To secure red-cell counts and cell-diameter determinations, two separate dilutions of blood were made in Hayem's solution and shaken for three minutes. Then a drop of each was spread on a Levy-Hauser counting chamber. The cells lying in 100 consecutive squares were counted, after which measurements of the red cells were made on the same slide, using a number 1-A cover slip to insure greater accuracy in measuring. Measurements of one diameter of 50 red blood cells were made for dogs 17 to 22, 30 to 32, and 36 and 37, by means of a calibrated Bausch and Lomb filar micrometer, using a magnification of 560 diameters. Readings were made to 0.2 micron. Since only one diameter was measured, it was necessary to select only round cells for measurement, which introduced a certain degree of selectivity into the measurements and therefore also a proportionate degree of inaccuracy. To determine the extent of error in sampling owing to measuring only 50 cells, duplicate sets of 50 cells each were measured on the same slide in a series of 10 consecutive slides. The maximum difference obtained was 0.14 micron, and the mean difference was 0.07 micron.

CHEMICAL MEASUREMENTS ON BLOOD

For determining the oxygen-combining capacity of the blood, the method described by Van Slyke (1917) and modified by Van Slyke and Neill (1924) was employed. For total and nonprotein nitrogen, the micro Kjeldahl method of Folin and Wu (1919), and the apparatus recommended by Parnas and Wagner (1921), with the modification suggested by Pregl (1924), were employed. In the short-term study, blood plasma was used for these determinations, whereas in the long-term study whole blood was used.

Determinations on the blood filtrate, prepared according to Folin's (1922) method for laked blood, included measurements of the nonprotein nitrogen by the micro Kjeldahl method; urea nitrogen, by Beattie's (1928) colorimetric method; preformed creatinine, by the

colorimetric method of Folin and Wu (1919); and amino acid nitrogen, by Van Slyke's (1913-14) gasometric method. The protein nitrogen of whole blood, as well as of blood plasma, was determined by the difference between the values for total nitrogen and nonprotein nitrogen.

For analyzing calcium and phosphorus, heparinized plasma was used. Although Holt (1931-32) reported appreciable amounts of calcium in heparin samples, and Kay and Byrom (1927) reported that heparin preparations contained organic phosphorus, the results of the present investigation did not confirm these observations. The addition of heparin to suitable inorganic salt solutions, in an amount equivalent to that used as the anticoagulant, did not influence the results obtained for either element.

Plasma proteins were precipitated by trichloracetic acid according to the method of Greenwald (1915, 1918). The oxidation of organic matter was carried out in an oil bath at 180° to 200° C., preliminary to the measurement of total phosphorus. The measurement of inorganic phosphorus was carried out on a trichloracetic acid filtrate, and the lipide phosphorus was extracted from plasma by the procedure of Bloor (1918). The colorimetric method of King (1932) was used for the phosphorus fractions. Both total and diffusible calcium were determined by the Roe and Kahn (1929) modification of Briggs' (1924) colorimetric method. The sample used for measuring diffusible calcium was obtained by the ultrafiltration of plasma for three hours through a collodion membrane at a pressure of 150 to 180 mm. of mercury. The nondiffusible calcium was calculated by difference after applying a correction for the volume occupied by plasma proteins (Smith and Sternberger, 1932).

For the determination of the plasma protein fractions, the fibrinogen was separated from the plasma, using the method of Wu (1922). A portion of this filtrate was used for the determination of the combined albumin and globulin content. The albumin fraction was determined on the filtrate after precipitating the globulin with sodium sulfate, using the method described by Howe (1921a,b). The globulins were determined by difference.

The filtrate for the determination of nonprotein nitrogen was prepared according to the method of Folin and Wu (1919).

Duplicate samples of all filtrates were digested, using Folin and Svedberg's (1930) digestion mixture, and the nitrogen content determined by the nesslerization technique of Folin (Folin and Denis, 1916; Folin and Wu, 1919). The Nessler's reagent employed was prepared according to the method of Koch and McMeekin (1924). After nesslerization the samples were read colorimetrically against the standard prepared from ammonium sulfate.

Mean corpuscular volume, in cubic microns, was calculated using the formula of Wintrobe (1931).

STATISTICAL METHODS

The data presented have been calculated and checked according to the formulas cited below.

To establish the degree of interdependence between various factors, correlation coefficients were calculated according to the formula:

$$r_{xy} = \frac{\Sigma\,(x\,y)\,/\,N - \bar{x}\,\bar{y}}{S_x\,S_y}$$

As a criterion of significance in the interpretation of the data, the following formula was used:

$$t = \frac{r\sqrt{n}}{\sqrt{1 - r^2}} \qquad (n = N - 2)$$

and the probability of "t" being exceeded solely through errors of random sampling was found in a suitable table constructed by Fisher (1928). In most instances, the 0.05 level of significance was considered satisfactory.

In a number of cases, it was desired to test the significance of the difference between two means. Where there was no possibility of a correlation existing between the two variables whose mean difference was being studied, Student's "t" test (Fisher, 1928) was employed, using the formula:

$$t = \frac{(\bar{x}\,\bar{y})\,\sqrt{n}}{\sqrt{N_x\,S_x^2 + N_y\,S_y^2}}\sqrt{\frac{N_x\,N_y}{N_x + N_y}}$$

where $n = N_x + N_y - 2$

with a final check upon the probability of "t" being exceeded solely through errors of random sampling.

Where the two variables were paired, thus introducing the possibility of a correlation existing between them, the standard error of the mean difference was determined from the formula:

$$SE_{\bar{d}} = \frac{S_d}{\sqrt{N - 1}}$$

the significance of this standard error being determined as described above after calculating:

$$t = \frac{\bar{d} - 0}{SE_{\bar{d}}}$$

III. NORMAL BLOOD FACTORS

IN EXPERIMENTS that involve a study of changes occurring in the blood, the investigator must have available information relative to the normal range of the various blood constituents. Only a few reports included in the literature cited contained reasonably complete information on large groups of animals. The data acquired in the present investigation include chemical and physical measurements on the blood of 50 experimental dogs (16 females, 34 males).

PHYSICAL MEASUREMENTS

In table 2 are presented data covering both male and female dogs. The values reported for individual animals are in most cases the means of two or more duplicate or triplicate determinations. Based on body weight, the mean total blood volume for these animals was 76.4 cc. per kilogram (70.9 cc. for 16 females and 79.0 cc. for 34 males). Comparison of the females' range with the males' reveals no significant sex difference. The total blood volume for the females, for example, ranged from 59.0 to 96.5 cc. per kilogram of body weight, as against 60.3 to 104.1 cc. per kilogram for the males. Similarly, cell volume per cent ranged from 37.7 to 59.0 per cent for the females as compared with 37.6 to 59.3 per cent for the males. Within both groups, however, individual differences were relatively large. Wide variations were apparent in red cell counts in millions per cubic millimeter of blood, ranging from 6.24 to 8.36 millions for the females, and from 5.08 to 8.55 millions for the males. These individual differences, therefore, emphasize the importance of securing normal values for each animal used in an investigation involving close observation of a changing blood picture.

Blood volume is frequently reported in terms of percentage of body weight. According to Peters and Van Slyke (1931), "The volume of the circulating blood of normal human beings and animals has been variously estimated by different observers using diverse methods, as from one-tenth to one-twentieth of the body weight." In the present study, the mean total blood volume of the 50 experimental animals, 76.4 cc per kilogram, was converted from volume of blood per kilogram to weight of blood per kilogram by using the specific gravity of blood (1.056) reported by Sherrington and Copeman (1893), producing a value of 72.3 g. per kilogram. In other words, blood constituted 7.23 per cent of the body weight of these experimental animals.

Table 3 covers data cited in the literature on normal dogs. A comparison drawn between these values and the results of the present investigation showed that the mean total blood volume observed for the dogs used in this study was lower than that reported by others who had

used the same method. Hooper, Smith, Belt, and Whipple (1920) using vital red as their dye, reported a mean total blood volume, for a group of 22 animals, of 101.3 cc. per kilogram of body weight. Camero and Krumbhaar (1933) reported a mean of 84.8 cc. per kilogram of body weight, while Harris (1920) obtained a mean of 84.1 cc. per kilogram using vital red dye, and 71.4 cc. per kilogram using Congo red. Powers, Bowie, and Howard (1930), using Congo red as their dye, reported a much higher figure, 112.8 cc. per kilogram, for a group of 25 dogs. The authors' results, using the dye method, coincide more closely with values reported by other investigators who used the carbon-monoxide method and do not agree with the high values reported by some workers using the dye method. Arnold, Carrier, Smith, and Whipple (1921), employing the carbon-monoxide method, reported a mean of 77.8 cc. per kilogram, while Plesch (1909) reported a mean of 79.5 cc., and Gréhant and Quinquad (1882) reported a mean of 81.6 cc. per kilogram. Investigators using the direct or Welcker method (Brodin, Richet, and Saint Girons, 1919-20, and Harris, 1920) apparently obtained the lowest values. The hemoglobin injection method produced values of 68.1 cc. per kilogram according to Harris (1920), 89.2 cc. per kilogram

Table 2. Physical Measurements on the Blood of 50 Normal Dogs (16 Females, 34 Males)

Dog No.	Weight in kg.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpus. volume, in cubic microns	White cell count, in thousands
Females (16)								
1	16.3	69.4	33.3	48.1	7.72	.....	62.3	6.04
2	12.0	77.9	38.2	50.0	8.07	.....	62.0	.....
3	13.2	66.5	31.5	47.2	7.99	.....	59.1	.....
4	11.9	62.8	23.6	37.7	.....	.....	.....	8.95
5	9.1	96.5	40.6	42.0	.....	7.13	.....	.....
6	19.0	76.0	40.8	54.2	.....	.....	.....	.....
15	14.1	68.2	30.6	44.8	7.02	.....	63.8	3.85
16	10.0	71.9	37.6	52.6	8.36	.....	62.9	5.55
19	17.9	71.1	42.0	59.0	7.27	6.85	81.2	4.07
20	17.6	71.7	29.8	41.5	6.24	.....	66.5	7.72
21	11.2	67.4	30.3	45.0	7.03	.....	64.0	9.37
22	12.4	59.0	26.6	45.1	6.77	.....	66.6	7.30
25	10.5	68.8	31.5	45.8	6.44	.....	71.1	8.70
26	14.7	65.8	28.8	43.7	6.66	.....	65.6	19.33
29	17.5	69.3	33.7	48.7	6.62	6.95	73.6	13.05
32	16.3	71.6	34.4	48.1	6.78	7.15	70.9	8.51
Mean	14.0	70.9	33.3	47.1	7.15	7.02	66.9	8.54
Median	14.1	69.4	33.3	47.2	7.02	7.13	65.6	8.51
Range	9.1-19.0	59.0-96.5	23.6-42.0	37.7-59.0	6.24-8.36	6.85-7.15	59.1-81.2	3.85-19.33

Table 2—Continued

Dog No.	Weight in kg.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpus. volume, in cubic microns	White cell count, in thousands
Males (34)								
7	15.4	79.0	39.4	49.8	8.55	.....	58.2	3.93
8	14.4	66.0	31.3	47.4	7.76	.....	61.1	5.50
9	12.7	66.6	25.9	38.9	5.09	7.02	76.4	7.30
10	15.9	67.1	35.8	53.4	7.01	6.98	76.2	16.17
11	12.7	85.4	33.6	39.3	5.38	7.13	73.0	.....
12	18.4	81.2	36.2	44.8	7.64	.....	58.6	.....
14	23.0	86.8	51.5	59.3	7.57	6.80	78.3	6.63
17	16.6	73.4	36.1	48.1	7.89	7.10	61.0	5.30
18	19.0	60.3	27.7	45.9	6.65	6.90	69.0	5.00
27	19.0	68.2	37.8	55.6	7.03	7.10	79.1	8.38
28	20.6	87.2	52.7	50.4	7.72	6.80	65.3	4.63
30	16.9	76.1	30.7	40.3	6.54	6.98	61.6	10.03
31	20.3	77.6	36.0	46.3	6.56	7.21	70.6	9.81
33	25.2	67.0	28.2	42.0	6.18	6.96	68.0	7.22
34	22.2	71.3	33.3	46.7	6.66	6.89	70.1	9.82
35	14.4	74.9	35.1	47.6	6.76	7.01	70.4	10.33
36	19.1	77.4	39.7	51.4	6.66	7.16	77.2	14.59
37	17.4	84.9	40.2	47.3	6.55	6.87	72.2	9.26
38	14.0	84.2	41.9	49.6	6.60	7.00	75.2	9.24
39	17.2	77.8	29.2	37.6	5.08	6.95	74.0	10.66
40	15.9	69.9	28.7	41.2	5.16	7.01	79.8	6.84
41	13.1	81.4	34.4	42.3	5.57	6.86	75.9	11.70
42	15.4	84.3	34.4	41.0	5.40	.....	75.9	11.23
43	14.6	88.6	36.7	41.4	5.14	.....	80.5	21.34
44	22.3	74.4	33.2	44.6	7.17	.....	62.2	6.75
45	18.0	82.0	42.6	52.1	7.43	.....	70.1	5.33
46	16.4	86.8	41.5	47.9	6.46	.....	74.2	9.65
47	17.1	90.5	46.5	51.4	6.78	.....	75.8	7.53
54	20.2	92.6	43.2	47.6	6.50	6.92	73.2	9.41
55	25.3	77.6	40.8	52.6	7.01	6.91	75.0	8.28
56	21.2	104.1	50.8	48.8	7.06	6.60	69.1	7.85
57	21.1	87.8	47.6	54.1	7.70	7.01	70.3	8.97
58	18.0	81.0	39.4	48.7	7.15	7.12	68.1	12.84
59	20.5	72.8	35.4	48.6	7.36	6.89	66.0	7.88
Mean	18.0	79.0	37.6	47.2	6.70	6.97	70.9	9.04
Median	18.0	77.8	36.1	47.6	6.76	6.96	72.2	8.38
Range	12.7-25.3	60.3-104.1	25.9-52.7	37.6-59.3	5.08-8.55	6.60-7.21	58.2-80.5	3.93-21.34
Mean, all dogs	16.7	76.4	36.2	47.2	6.83	6.98	69.8	8.91

according to Franke and Benedict (1920-21), and 105.3 cc. per kilogram according to Lee and Whipple (1921). Attention is directed to the wide range of values obtained by nearly all of the different methods.

Table 3. Physical Measurements on the

	Reference	No. of dogs	Method for blood volume	Total blood vol. in cc. per kg.	
				Range	Mean
1	This study	29-50	Vital Red	59.0-104.1	76.4
2	Gibson et al. (1938)	50	Spectrophotometric Blue Dye	84.0- 97.3	92.7†
3	Bruner & Wakerlin (1937)	34		.....	.....
4	Weech et al. (1937)	60	Dye‖	.....	92.9¶
5	Wintrobe et al. (1935-36)	54		.....	.....
6	Ashley & Guest (1934)	50		.....	.....
7	Camero & Krumbhaar (1933)	6	Vital Red	.....	84.8†
8	Mayerson (1930)	60		.....	.....
9	Powers et al. (1930)	25	Congo Red	83.0-136.0	112.8
10	Kohanawa (1928)	12		.....	.....
11	Pierce & Plant (1928)	16		.....	.....
12	Emmons (1927-28)	.....		.....	.....
13	Klieneberger (1927)	6		.....	.....
14	Bodansky & Dressler (1927)	8		.....	.....
15	Bennett (1926)	7		.....	.....
16	Gram (1924)	3		.....	.....
17	Lee & Whipple (1921)	9	Hemoglobin	100.4-114.2	105.3
18	Arnold et al. (1921)	4	Carbon Monoxide	72.1- 89.7	77.8
19	Whipple & Robscheit (1921)	7	Brilliant Vital Red	87.0-132.0	109.3†
20	Franke & Benedict (1920-21)	19	Hemoglobin	77.2-109.0	89.2†
21	Harris (1920)	8	Direct	59.0- 72.7	65.4¶
22	Harris (1920)	6	Hemoglobin	60.8- 74.6	68.1¶
23	Harris (1920)	4	Vital Red	78.8- 88.3	84.1¶
24	Harris (1920)	6	Congo Red	66.2- 76.9	71.4¶
25	Whipple, Hooper & Robscheit (1920)	12	Vital Red Dry Oxalate	72.0-146.0	115.0†
26	Whipple, Hooper & Robscheit (1920)	15	Vital Red Isotonic Oxalate	89.0-147.0	108.3†
27	Hooper, Smith et al. (1920)	22	Vital Red	83.7-115.3	101.3
28	Brodin et al. (1919-20)	60	Direct	.....	64.4‡
29	Gasser et al. (1919)	.....		.....	.....
30	Kuhl (1919)	10		.....	.....
31	Meek & Gasser (1918-19)	8	Gum Acacia	78.4-107.8	97.4
32	Brodin et al. (1918)	29		61.0- 73.0	67.4
33	Wells & Sutton (1915-16)	.....		.....	.....
34	Mann (1915)	6	Direct (without washout)	54.0- 67.0¶	59.2†
35	Lamson (1915)	.....		.....	.....
36	Musser & Krumbhaar (1914)	47		.....	.....
37	Nassau (1913-14)	35		.....	.....
38	Musser & Krumbhaar (1913)	5-17		.....	.....
39	Magnan (1911)	.....		.....	.....
40	Goodall (1910)	.....		.....	.....
41	Lenze (1909)	.....		.....	.....
42	Nelson (1909)	2	Dilution	67.4- 67.4	67.4¶
43	Plesch (1909)	5	Carbon Monoxide	70.6- 90.0	79.5¶
44	Burnett & Traum (1905)	6-7		.....	.....
45	Breuer & v Seiller (1903)	3		.....	.....
46	Busch & Van Bergen (1902)	20		.....	.....
47	Biedl & v Decastello (1901)	.....		.....	.....
48	Courmont & Lesieur (1901)	20		.....	.....
49	Dawson (1900-01)	12		.....	.....
50	Schauman & Rosenqvist (1898)	1		.....	.....
51	Sherrington (1894)	.....		.....	.....
52	Sussdorf (1890)	.....		.....	.....
53	Hayem (1889)	20		.....	.....
54	Formad (1888)	.....		.....	.....
55	Ellenberger (1887)	.....		.....	.....
56	Gréhant & Quinquad (1882)	9	Carbon Monoxide	72.3- 91.6	81.6¶
57	Schmidt (1878)	.....		.....	.....
58	Manassein (1872)	.....		.....	.....
59	Welcker (1863-64)	.....		.....	.....
60	Vierordt (1854)	.....		.....	.....
61	Schmidt (1848)	.....		.....	.....

* Calculated from total cell volume and weight. † Calculated average. ‡ Calculated from weight. ‖ Dye not given. ** Forty-three dogs.

Blood of Normal Dogs Cited in the Literature

Cell volume in cc. per kg.		Cell volume per cent		Red cell count, in millions		Mean red cell diam. in microns	Mean corp. vol. in cubic microns	White cell count, in thousands		
Range	Mean	Range	Mean	Range	Mean			Range	Mean	
23.6-52.7	36.2	37.6-59.3	47.2	5.08- 8.55	6.83	6.98	69.8	3.85-21.34	8.91	1
36.4-54.6	43.8†	38.1-60.0	47.8†	.....	.....	.....	.....	.....	.....	2
.....	.....	.....	44.3	.....	6.45	.....	68.9	.....	14.18	3
.....	44.5*	.....	47.5	.....	.....	.....	.....	.....	.....	4
.....	.....	.....	47.3	.....	7.02	.....	67.6	.....	.....	5
.....	.....	.....	46.9	.....	6.87	.....	66.6	.....	.....	6
.....	40.2†	25.0-49.0	40.9**	4.09- 8.33	6.95†	.....	.....	.....	.....	7
.....	.....	.....	38.6	4.86- 8.56	6.16	.....	59.3	5.65-17.35	11.16	8
39.6-61.3	50.9*	34.4-50.6	45.2	4.63- 8.61	7.00	.....	.....	.....	.....	9
.....	.....	.....	.....	5.24- 7.16	6.21	6.80	.....	12.80-19.80	17.10	10
.....	.....	.....	.....	5.40- 6.73	.....	.....	.....	8.70-17.90	.....	11
.....	.....	.....	43.0	.....	6.25	7.20	69.0	.....	.....	12
.....	.....	.....	.....	6.00- 9.76	7.22	6.80	.....	5.70-11.10	10.00	13
.....	.....	.....	.....	7.16-10.50	8.47†	.....	50.7	.....	.....	14
.....	.....	31.0-43.0	38.0†	.....	.....	.....	.....	.....	.....	15
.....	.....	33.7-52.0	43.7†	.....	.....	.....	.....	.....	.....	16
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	17
36.8-45.7	42.0	43.0-63.4	51.5	.....	.....	.....	.....	.....	.....	18
42.4-73.3*	56.9†	45.6-59.4	51.7†	6.90-11.80	8.29†	.....	.....	7.00-13.40	9.98†	19
.....	.....	24.0-45.2	36.0†	.....	.....	.....	.....	.....	.....	20
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	21
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	22
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	23
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	24
35.7-82.2*	62.0†	45.0-60.0	53.1†	6.60-10.10	7.79†	.....	.....	6.20-15.80	9.79†	25
40.3-89.5*	59.9†	45.5-62.6	55.1†	6.70-11.40	8.77†	.....	.....	6.20-14.80	10.52†	26
29.1-69.2*	52.5†	34.4-63.5	51.6	.....	.....	.....	.....	.....	.....	27
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	28
.....	.....	.....	.....	5.19- 8.17	6.68†	.....	.....	.....	.....	29
.....	.....	.....	.....	.....	6.59	.....	.....	.....	12.60	30
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	31
.....	.....	.....	.....	6.00- 8.00	6.90§	.....	.....	10.00-19.00	14.50	32
.....	.....	.....	.....	.....	6.71	.....	.....	.....	11.00	33
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	34
.....	.....	.....	.....	6.74- 8.00	7.46†	.....	.....	.....	.....	35
.....	.....	.....	.....	4.63- 7.76	5.97	.....	.....	8.80-33.05	15.92	36
.....	.....	.....	.....	5.40- 7.30	6.40	.....	.....	5.00-13.40	7.70	37
.....	.....	.....	.....	4.61- 5.52	5.20†	.....	.....	9.00-14.40	12.52†	38
.....	.....	.....	.....	.....	.....	7.00	.....	.....	.....	39
.....	.....	.....	.....	.....	5.60	7.00	.....	11.20-31.20	19.50	40
.....	.....	.....	.....	.....	.....	7.65	.....	.....	.....	41
.....	.....	.....	.....	5.17- 5.94	5.56†	.....	.....	.....	.....	42
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	43
.....	.....	.....	.....	5.72- 6.56	6.03†	.....	.....	5.86- 8.89	7.60†	44
.....	.....	.....	.....	6.90- 7.95	7.42†	.....	.....	.....	.....	45
.....	.....	.....	.....	4.23- 8.03	6.21	.....	.....	7.20-14.38	9.53	46
.....	.....	.....	.....	.....	.....	.....	.....	.....	12.00	47
.....	.....	.....	.....	.....	.....	.....	.....	6.00-10.00	.....	48
.....	.....	.....	.....	6.25- 8.50	7.21	7.00	.....	11.00-28.00	19.30	49
.....	.....	.....	.....	.....	.....	6.95	.....	.....	.....	50
.....	.....	.....	.....	2.50- 8.13	5.58†	.....	.....	6.26-15.90	9.40†	51
.....	.....	.....	.....	.....	.....	7.30	.....	.....	.....	52
.....	.....	.....	.....	.....	6.65	7.20	.....	.....	.....	53
.....	.....	.....	.....	.....	.....	7.00	.....	.....	.....	54
.....	.....	.....	.....	.....	.....	7.30	.....	.....	.....	55
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	56
.....	.....	.....	.....	.....	.....	6.40	.....	.....	.....	57
.....	.....	.....	.....	.....	.....	6.95	.....	.....	.....	58
.....	.....	.....	.....	.....	.....	7.30	.....	.....	.....	59
.....	.....	.....	.....	.....	.....	7.30	.....	.....	.....	60
.....	.....	.....	.....	.....	.....	7.00	.....	.....	.....	61

grams per 100 grams to cc. per kg. §Forty-five dogs. ¶ Calculated from total blood volume and

Table 4. Differential White Cells Counts on the

	Reference	No. of dogs	Neutrophiles, per cent		Small lymphocytes, per cent		Large lymphocytes, per cent	
			Range	Mean	Range	Mean	Range	Mean
1	This study	5	78.9-87.1	82.6	2.2-15.8	9.3	1.0- 4.8	2.5
2	Mayerson (1930)	60	56.0-89.0	74.0	9.0-34.0	20.0	0.0-10.0	4.0
3	Pierce & Plant (1928)	16	69.0-75.0	.....	19.0-20.0	.....	5.0- 7.0	.....
4	Kohanawa (1928)	12	63.4-84.8	77.4	6.6-22.7	10.7	1.4- 3.9	2.5
5	Klieneberger (1927)	6	49.0-88.0	77.4	21.0-43.6	27.4*	0.5- 3.4	1.7*
6	Kuhl (1919)	10	.....	57.0	.....	25.0	.....	8.0
7	Burket (1917)	2	43.1-64.0	55.2*	.....	.....	.....	.....
8	Mann (1916)	6	64.0-79.0	73.0	5.0-20.0	11.0*	6.0-13.0	10.0*
9	Musser & Krumbhaar (1914)	22	40.5-81.0	66.6	10.0-51.0	22.1	1.0-17.5	6.8
10	Nassau (1913-14)	35	63.0-79.0	72.0	.....	.....	.....	.....
11	Furno (1913)	11	52.0-84.5	69.2*	.....	.....	.....	.....
12	Schittenhelm et al. (1911-12)	15	39.0-82.0	71.8	.....	.....	.....	.....
13	Goodall (1910)	.....	60.0-78.0	63.0	.....	.....	.....	.....
14	Burnett & Traum (1905)	7	57.9-76.1	68.4	.....	.....	.....	.....
15	Selinoff (1904)	20	69.4-87.2	79.6*	.....	.....	.....	.....
16	Reckzeh (1904)	4	70.0-80.0	75.0*	8.5-16.0	13.9*	3.0- 4.0	3.6*
17	Goodall et al. (1903-04)	1	.....	60.5†	.....	.....	.....	.....
18	Nicolas & Dumoulin (1903)	2	.....	72.5*	.....	.....	.....	.....
19	Busch & Van Bergen (1902)	20	54.3-87.5	65.7	.....	.....	.....	.....
20	Biedl & v Decastello (1901)	.....	.....	75.0	.....	.....	.....	5.8
21	Courmont & Lesieur (1901)	20	52.0-85.0	69.0	.....	.....	.....	.....
22	Dawson (1900-01)	12	62.4-68.0	64.6	.....	.....	.....	.....
23	Tallqvist & Willebrand (1899-1900)	15	68.4-83.8	75.1*	.....	.....	.....	.....
24	Zenoni (1894)	.....	.....	62.0	.....	.....	.....	.....

* Calculated average.
† Calculated from number per cu. mm.
‡ Includes transitional cells.

The mean cell volume per cent noted in the present research, 47.2 per cent, coincides well with the median of the values found in the literature. The wide range of normal values for red cell count reported by various investigators is in agreement with the authors' observation that normal animals may differ markedly in this respect. The mean erythrocyte count noted in the present investigation, 6.83 millions per cubic millimeter, was slightly above the median for the dogs cited in the literature. The mean diameter of red blood cells for the dogs used in this investigation, based on measurements for 30 animals, was 6.98 microns. Burnett (1917), in summarizing the work of six investigators, reported a range of 6.95 to 7.30 microns. Kohanawa (1928) listed a slightly lower figure, 6.80 microns, while Emmons (1927-28) reported a value of 7.20 microns. These differences can probably be explained, at least in part, by the methods used for securing the measurements.

Few workers have included in their reports data on mean corpuscular volume. With the exception of the low value reported by Bodansky and Dressler (1927), based on seven animals, the values included in the

Blood of Normal Dogs Cited in the Literature

Total lymphocytes, per cent		Monocytes, per cent		Eosinophiles, per cent		Basophiles, per cent	
Range	Mean	Range	Mean	Range	Mean		
7.0-16.8	11.8	0.8- 3.8	2.1	0.5- 6.8	2.8	0.0	1
.....	24.0	.....	.....	0.0- 7.0	2.0	Occasionally	2
.....	.....	.....	.....	1.0- 3.0	.....	0.0-1.0	3
.....	13.2	.....	6.1	1.6- 7.8	3.3	0.0	4
.....	.....	.....	.....	3.0-13.0	4.2	Rare	5
.....	33.0	.....	.....	.....	10.0	1.0	6
11.5-20.4	16.9*	0.9- 2.4	1.8*	9.3-24.4	18.6*	0.3*	7
.....	.....	1.0- 6.0‡	3.5*	0.0- 7.0	4.0*	.....	8
.....	.....	.....	.....	0.0-20.0	5.1	.....	9
13.0-25.5	18.6	2.6- 6.5‡	5.2	.....	.....	Very seldom	10
.....	.....	.....	.....	0.5- 2.6	1.5*	4.1	11
12.0-55.0	18.5	1.5- 9.5‡	4.1	2.0-11.0	3.7	.....	12
20.0-35.0	30.0	.....	.....	2.0-14.0	7.0	.....	13
11.0-28.0	17.6	3.1-10.4	6.4	3.7-10.5	7.3	.....	14
6.6-16.6	12.7*	2.8- 9.4	6.1*	.....	.....	.....	15
.....	.....	0.0- 0.5	0.1	0.0- 5.0	2.2*	.....	16
.....	25.0†	.....	.....	.....	14.5†	.....	17
.....	10.3*	.....	17.8*	.....	3.3*	.....	18
3.7-35.0	21.0	3.7-13.2	6.8	0.8-15.2	5.3	.....	19
.....	15.8	.....	.....	.....	3.4	.....	20
.....	.....	.....	.....	.....	.....	.....	21
11.2-31.6	22.2	.....	.....	2.6-21.6	8.8	.....	22
4.2-10.8	6.2*	9.6-17.4	13.5*	0.2- 8.1	5.3*	.....	23
.....	.....	.....	.....	.....	10.0	.....	24

literature fall within the range noted for the present experiments. The lowest mean corpuscular volume observed was 58.2 cubic microns; the highest, 81.2 cubic microns; the mean for all of the dogs, 69.8 cubic microns. There was no apparent difference between the males and females with respect to mean corpuscular volume.

Considerable variation was observed in the leucocyte counts of the different animals. The females' counts ranged from 3.85 to 19.33 thousands per cubic millimeter; the males, from 3.93 to 21.34 thousands. The group mean, 8.91 thousands, agrees well with the mean normal figure, 8.00 thousands, suggested by Burnett. The means reported by Kohanawa (1928), 17.10 thousands; by Musser and Krumbhaar (1914), 15.92 thousands; by Dawson (1900-01), 19.30 thousands; and by Goodall (1910), 19.50 thousands appear to be unusually high.

The inclusion of data on 18 additional male dogs did not alter significantly the mean values reported earlier for physical or chemical measurements on blood of normal dogs (Leichsenring, Biester, *et al.*, 1932).

Table 5. Differential White Cell Counts on the Blood of Normal Dogs (5 Males)

Dog No.	Neutrophiles, per cent				Lymphocytes, per cent			Monocytes, per cent	Eosinophiles, per cent	Basophiles, per cent
	Immature	Young band forms	Mature segmented	Total	Small	Large	Total			
39	0.2	14.8	66.7	81.7	15.8	1.0	16.8	0.8	0.5	0.2
40	0.3	16.8	70.0	87.1	2.2	4.8	7.0	2.2	3.5	0.0
41	0.0	23.0	60.6	83.6	7.2	3.8	11.0	3.8	2.0	0.0
42	0.9	11.1	66.9	78.9	9.4	1.5	10.9	2.0	6.8	0.0
43	1.7	17.0	63.1	81.8	11.9	1.5	13.4	1.7	1.1	0.0
Mean	0.6	16.5	65.5	82.6	9.3	2.5	11.8	2.1	2.8	0.0
Median	0.3	16.8	66.7	81.8	9.4	1.5	11.0	2.0	2.0	0.0
Range	0.0-1.7	11.1-23.0	60.6-70.0	78.9-87.1	2.2-15.8	1.0-4.8	7.0-16.8	0.8-3.8	0.5-6.8	0.0-0.2

Table 5 shows the results of the differential white cell count for the five male animals on which this determination was made. The dogs involved showed a somewhat higher mean percentage of neutrophiles and a lower percentage of lymphocytes and monocytes than have been reported by other investigators (table 4).

CHEMICAL MEASUREMENTS

Tables 6 and 7 give data on various whole blood and plasma measurements. As with physical measurements, the values reported in most cases represent the mean of two or more duplicate or triplicate determinations. Evidently there was no essential difference, owing to sex, in the levels of the various constituents of whole blood. For both groups, widely ranging values were observed for the oxygen-combining capacity of the blood in volumes per cent. For the females, these values ranged from 13.2 to 22.9 volumes per cent; for the males, from 11.2 to 24.1 volumes per cent. The females' mean, 17.8 volumes per cent, was nearly identical with the males' mean, 17.4 volumes per cent. Total nitrogen in the blood was in a large degree influenced by the hemoglobin level. The means, medians, and ranges for the males and females were quite similar. The nonprotein nitrogen of whole blood also varied considerably, animal to animal, from 0.030 to 0.048 g. per 100 cc. for the females, with a mean of 0.036 gram; and from 0.020 to 0.042 g. for the males, with a mean of 0.034 gram. Although few data, covering urea nitrogen, amino acid nitrogen, and creatinine in whole blood were secured for the females, there appeared to be no sex difference in the levels of these particular blood constituents.

Table 6 shows a somewhat lower mean for oxygen-combining capacity for the animals used in the present investigation than values reported by most workers whose data are cited in the literature (table 8), although the mean of 17.5 volumes per cent is almost identical with that reported by Mayerson (1930) for his 60 canine subjects (17.4 volumes per cent). Except for the study by Van Slyke and Meyer (1913-14b), the literature offered no data covering the total nitrogen content of the blood. Data resulting from the present investigation seem to be in fair accord with values found in the literature for the nonprotein nitrogenous constituents considered.

In the consideration of the various plasma constituents, seven male dogs were observed. Table 7 presents the data acquired. Tables 9 and 10 were prepared to show the data reported in the literature covering the same plasma constituents and to permit comparison with the results of this research. It will be noted that there was no marked disagreement between the means obtained in the present investigation and those reported in the literature, and the ranges for the various values were also of the same magnitude.

Table 6. Chemical Measurements on the Blood of Normal Dogs (13 Females, 33 Males)

Dog No.	Oxygen-combining capacity, in volumes per cent	Total nitrogen in whole blood, g. per 100 cc.	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	Amino acid nitrogen in whole blood, mg. per 100 cc.	Creatinine in whole blood, mg. per 100 cc.
Females							
1	21.2	.....	.....	.....	.....	.....	.....
2	21.6	.....	.....	.....	.....	.....	.....
3	21.4	.....	.....	.....	.....	.....	.....
15	18.3	2.89	0.039	2.85	.....	.....	.....
16	14.9	3.23	0.048	3.18	.....	.....	.....
19	22.9	3.50	0.034	3.46	15.38	4.05	1.72
20	13.2	2.89	0.030	2.86	.....	.....	.....
21	14.1	3.18	0.030	3.15	.....	.....	.....
22	15.7	3.03	0.039	2.99	.....	.....	.....
25	15.6	3.22	0.032	3.19	.....	.....	.....
26	15.2	3.06	0.041	3.02	.....	.....	.....
29	18.9	3.16	0.030	3.13	16.50	11.58	1.54
32	17.8	3.27	0.039	3.23	7.41	.....	.....
Mean	17.8	3.14	0.036	3.11	13.10	7.82	1.63
Median	17.8	3.18	0.039	3.15	15.38	.....	.....
Range	13.2-22.9	2.89-3.50	0.030-0.048	2.85-3.46	7.41-16.50	4.05-11.58	1.54-1.72
Males							
7	21.4	.....	.....	.....	.....	.....	.....
8	20.0	.....	.....	.....	.....	.....	.....
9	13.1	3.00	0.036	2.96	.....	.....	.....
10	21.4	3.48	0.035	3.45	.....	3.37	.....
12	20.2	.....	.....	.....	.....	.....	.....
14	21.8	3.82	0.031	3.79	4.96	.....	1.34
17	18.6	3.42	0.029	3.39	9.34	4.84	1.47
18	17.4	3.05	0.029	3.03	10.34	.....	1.65
27	19.4	3.25	0.036	3.21	14.71	10.44	1.43
28	24.1	3.52	0.020	3.50	6.26	18.68	1.36
30	17.4	3.14	0.042	3.09	7.87	.....	.....
31	16.4	3.36	0.042	3.32	7.88	.....	.....
33	13.2	2.91	0.035	2.87	14.25	.....	.....
34	17.0	3.16	0.033	3.13	12.29	.....	.....
35	16.2	3.12	0.041	3.08	8.95	.....	.....
36	18.5	.....	.....	.....	.....	.....	.....
37	18.0	.....	.....	.....	.....	.....	.....
38	18.0	.....	.....	.....	.....	.....	.....
39	13.8	.....	.....	.....	.....	.....	.....
40	14.6	.....	.....	.....	.....	.....	.....
41	15.5	.....	.....	.....	.....	.....	.....
42	11.7	.....	.....	.....	.....	.....	.....
43	11.2	.....	.....	.....	.....	.....	.....
44	16.1	.....	.....	.....	.....	.....	.....
45	18.1	.....	.....	.....	.....	.....	.....
46	19.0	.....	.....	.....	.....	.....	.....
47	17.1	.....	.....	.....	.....	.....	.....
54	18.3	.....	.....	.....	.....	.....	.....
55	18.5	.....	.....	.....	.....	.....	.....
56	16.7	.....	.....	.....	.....	.....	.....
57	18.0	.....	.....	.....	.....	.....	.....
58	14.9	.....	.....	.....	.....	.....	.....
59	18.4	.....	.....	.....	.....	.....	.....
Mean	17.4	3.27	0.034	3.24	9.68	9.33	1.45
Median	18.0	3.25	0.035	3.21	9.34	10.44	1.43
Range	11.2-24.1	2.91-3.82	0.020-0.042	2.87-3.79	4.96-14.71	3.37-18.68	1.34-1.65
Mean, all dogs	17.5	3.21	0.035	3.18	10.47	8.83	1.50

Table 7. Chemical Measurements on Plasma of Normal Dogs (7 males)

Dog No.	Total nitrogen in plasma, g. per 100 cc.	Non-protein nitrogen in plasma, g. per 100 cc.	Protein nitrogen in plasma, g. per 100 cc.	Fibrin in plasma, g. per 100 cc.	Albumin in plasma, g. per 100 cc.	Globulin in plasma, g. per 100 cc.	Total phosphorus in plasma, mg. per 100 cc.	Lipide phosphorus in plasma, mg. per 100 cc.	Inorganic phosphorus in plasma, mg. per 100 cc.	Total calcium in plasma, mg. per 100 cc.	Diffusible calcium in plasma, mg. per 100 cc.	Nondiffusible calcium in plasma, mg. per 100 cc.
Males												
44	1.104	0.024	1.080	0.26	3.91	2.26	25.9	17.1	6.01	13.46	8.52	4.98
45	1.075	0.031	1.044	0.24	3.84	2.01	24.7	19.9	5.02	12.92	8.71	4.28
46	1.269	0.032	1.237	0.28	3.41	2.89	21.4	12.8	6.36	13.46	8.46	5.05
47	1.103	0.037	1.066	0.47	4.60	2.36	21.9	16.7	4.14	10.18	6.75	4.92
54	.....	0.033	.....	0.30	3.88	2.02	.....	.....	.....	.....	.....	.....
55	.....	0.029	.....	0.17	3.68	2.44	.....	.....	.....	.....	.....	.....
56	.....	0.031	.....	0.28	4.25	3.10	.....	.....	.....	.....	.....	.....
Mean	1.138	0.031	1.107	0.29	3.94	2.44	23.5	16.6	5.38	12.50	8.11	4.81
Median	1.104	0.031	1.080	0.28	3.88	2.36	24.7	17.1	6.01	13.46	8.52	4.98
Range	1.075-1.269	0.024-0.037	1.044-1.237	0.17-0.47	3.41-4.60	2.01-3.10	21.4-25.9	12.8-19.9	4.14-6.36	10.18-13.46	6.75-8.71	4.28-5.05

Table 8. Chemical Measurements on the Blood of Normal Dogs Cited in the Literature

	Reference	No. of dogs	Oxygen-combining capacity, in volumes per cent		Total nitrogen in whole blood, in g. per 100 cc.		Nonprotein nitrogen in whole blood, in g. per 100 cc.		Urea nitrogen in whole blood, mg. per 100 cc.		Amino acid nitrogen in whole blood, mg. per 100 cc.		Creatinine in whole blood, mg. per 100 cc.		
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
1	This study	6-46	11.2-24.1	17.5	2.89-3.82	3.21	0.020-0.048	0.035	4.96-16.50	10.47	3.37-18.68	8.83	1.34-1.72	1.50	1
2	Gibson et al. (1938)	18	16.8-24.7	20.2†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2
3	Bruner & Wakerlin (1937)	34	.....	18.2†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
4	Wintrobe et al. (1935-36)	54	.....	19.6†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4
5	Ashley & Guest (1934)	43	.....	21.4†	.....	.....	.....	0.035	.....	.....	.....	.....	.....	.....	5
6	Cahn & Houget (1933)	35	.....	.....	.....	.....	.....	.....	3.7 -12.6§	.....	.....	.....	.....	.....	6
7	Camero & Krumbhaar (1933)	6	12.0-24.4	20.0‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7
8	Mayerson (1930)	60	.....	17.4†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
9	Powers et al. (1930)	25	13.8-27.2	21.2‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	9
10	Stucky & Rose (1929)	10	15.2-23.6	20.2‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	10
11	Teel & Watkins (1929)	2	.....	.....	.....	.....	0.028-0.042	0.036*	.....	.....	.....	.....	1.2 -1.5	1.3*	11
12	Pierce & Plant (1928)	16	16.2-20.7‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	12
13	Emmons (1927-28)	.....	.....	20.8‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	13
14	Arnold & Mendel (1927)	3	.....	.....	.....	.....	0.035-0.040	0.037*	.....	.....	.....	.....	.....	.....	14
15	Klieneberger (1927)	6	21.2-33.9	25.4‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15
16	Collip et al. (1925)	4	.....	.....	.....	.....	0.026-0.043	0.038*	10.0 -16.0	13.5*	.....	.....	.....	.....	16
17	Haden & Orr (1925)	200	.....	.....	.....	.....	0.016-0.078	0.031	5.1 -42.1	11.7	2.0 -14.6	6.7¶	0.5 -2.6	1.5	17
18	Fisher & Larson (1925)	5	.....	.....	.....	.....	0.038-0.049	0.040*	.....	.....	.....	.....	.....	.....	18
19	Seth & Luck (1925)	1	.....	.....	.....	.....	.....	.....	.....	7.7	.....	5.9	.....	.....	19
20	Morgulis & Edwards (1924)	6	.....	.....	.....	.....	0.032-0.040	0.035*	11.1 -15.1	12.9*	7.8 - 9.1	8.7*	1.5 -1.7	1.6*	20
21	Haden & Orr (1923)	25	.....	.....	.....	.....	0.020-0.052	0.030	5.1 -26.1	11.1	4.9 - 7.7	6.3	1.1 -2.1	1.5	21
22	Haden & Orr (1923)	6	.....	.....	.....	.....	0.022-0.039	0.031*	7.0 -23.4	13.1*	5.3 - 8.1	6.6*	1.2 -1.6	1.3*	22
23	Scheunert & v. Pelchrzim (1923)	2	.....	.....	.....	.....	.....	.....	11.1 -16.7	14.1*	.....	.....	1.2 -1.5	1.3*	23
24	Okada & Hayashi (1922)	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.3 - 8.8	7.3	.....	.....	24
25	Whipple & Robscheit (1921)	7	19.6-25.0	22.7‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	25

Table 8—Continued

	Reference	No. of dogs	Oxygen-combining capacity, in volumes per cent		Total nitrogen in whole blood, in g. per 100 cc.		Nonprotein nitrogen in whole blood, in g. per 100 cc.		Urea nitrogen in whole blood, mg. per 100 cc.		Amino acid nitrogen in whole blood, mg. per 100 cc.		Creatinine in whole blood, mg. per 100 cc.		
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
26	Austin et al. (1921)	2	.....	.....	.....	.....	.....	.....	9.2 -25.6	13.8*	.....	.....	.....	.....	26
27	Whipple, Hooper & Robscheit (1920)	12	22.1-34.6	27.9‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	27
28	Whipple, Hooper & Robscheit (1920)	15	15.5-30.7	23.9‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	28
29	Fritsch (1920)	10	.....	21.2†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	29
30	Kuhl (1919)	10	.....	21.2†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	30
31	Bock (1917)	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.7 - 8.4	7.5	.....	.....	31
32	Draper (1917)	1	.....	.....	.....	.....	.....	0.044	.....	18.0	.....	.....	.....	0.1	32
33	Wilson & Plass (1917)	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.7	33
34	Bang (1915-16)	.....	.....	.....	.....	.....	.....	.....	17.0 -27.0	.....	8.0 -18.0	.....	.....	.....	34
35	György & Zunz (1915)	27	.....	.....	.....	.....	.....	.....	.....	.....	4.0 - 5.8	4.7*	.....	.....	35
36	Taylor & Lewis (1915)	4	.....	.....	.....	.....	0.018-0.030	0.026*	6.9 -22.6	12.8*	.....	.....	.....	.....	36
37	Marshall & Davis (1914)	1	.....	.....	.....	.....	.....	.....	12.6 -13.1§	12.8*,§	.....	.....	.....	.....	37
38	Musser & Krumbhaar (1914)	47	17.2-23.3	20.8‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	38
39	Shaffer (1914)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.8 -1.6	1.2*	39
40	Van Slyke & Meyer (1912)	8	.....	.....	.....	.....	.....	.....	.....	.....	3.1 - 5.4	4.4*	.....	.....	40
41	Van Slyke & Meyer (1913-14a)	3	.....	.....	.....	.....	.....	.....	.....	.....	3.9 - 5.9	5.1*	.....	.....	41
42	Van Slyke & Meyer (1913-14b)	5	.....	.....	3.32-3.99	3.47*	.....	.....	5.0 -14.0	9.3*	3.9 -10.0	6.1*	.....	.....	42
43	Costantino (1913)	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.2	.....	.....	43
44	Furno (1913)	24	14.3-25.4	19.2‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	44
45	Plesch (1909)	5	19.3-23.8	21.3*	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	45
46	Breuer & v Seiller (1903)	3	23.6-26.4	25.2†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	46
47	Dawson (1900-01)	12	12.5-20.8	15.9‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	47
48	Otto (1885)	17	16.2-21.4	18.8†	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	48

* Calculated average. † Calculated from grams of hemoglobin per 100 cc. of blood. ‡ Calculated from percentage hemoglobin. § Calculated from grams of urea per 100 cc. of blood. ¶ One hundred dogs.

Table 9. Chemical Measurements on Plasma of Normal Dogs Cited in the Literature (Nitrogenous Constituents)

	Reference	No. of dogs	Nonprotein nitrogen in plasma, g. per 100 cc.		Protein nitrogen in plasma, g. per 100 cc.		Fibrin in plasma, g. per 100 cc.		Albumin in plasma, g. per 100 cc.		Globulin in plasma, g. per 100 cc.		
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
1	This study	4-7	0.024-0.037	0.031	1.044-1.237	1.107	0.17-0.47	0.29	3.41-4.60	3.94	2.01-3.10	2.44	1
2	McNaught et al. (1936)	12	.....	.....	0.790-1.240‡	0.930‡	.....	.....	3.18-4.28	3.74	1.46-3.50	2.05	2
3	Weech et al. (1935)	19	.....	.....	.....	1.018‡	.....	.....	.....	3.38	.....	2.98	3
4	Turner & Gibson (1932)	4	.....	.....	0.930-1.050‡	.....	.....	.....	.....	.....	.....	.....	4
5	Moore & Stewart (1930-31)	1	.....	.....	.....	0.850‡	.....	.....	.....	2.94	.....	2.40	5
6	Vars (1930)	1	.....	.....	.....	.....	0.38-0.42	0.40*	.....	.....	.....	.....	6
7	Matthew (1927)	50	.....	.....	1.080-1.310	1.180†,‡	0.34-0.70‡	0.46‡	4.23-5.91‡	4.75‡	1.82-2.97‡	2.17‡	7
8	Atkinson & Ets (1922)	6	0.021-0.038	0.028	.....	.....	.....	.....	.....	.....	.....	.....	8
9	Foster & Whipple (1921-22)	13	.....	.....	.....	.....	0.31-0.51	0.39	.....	.....	.....	.....	9
10	Smith et al. (1920)	10	.....	.....	.....	.....	0.17-0.77‡,‖	0.34‡,‖	3.28-4.61‡,¶	3.78*,‡,¶	1.33-2.97‡,¶	1.84*,‡,¶	10
11	Whipple (1914)	11	.....	.....	.....	.....	0.20-0.87	0.47	.....	.....	.....	.....	11
12	Morawitz (1906)	7	.....	.....	0.780-0.980§	0.890†,§	.....	.....	3.31-3.83	3.55*,†	1.28-2.71	1.99*,‡	12
13	Lewinski (1903)	.....	.....	.....	.....	0.960†	.....	0.60	.....	3.17	.....	2.26	13

* Calculated average.
† Protein nitrogen calculated from gms. of protein per 100 cc. plasma.
‡ Calculated from percentage.
§ Calculated from percentage protein in plasma.
¶ Determinations made on serum.
‖ Twenty dogs.

Table 10. Chemical Measurements on Plasma of Normal Dogs Cited in the Literature (Phosphorus and Calcium Fractions)

	Reference	No. of dogs	Total phosphorus in plasma, mg. per 100 cc.		Lipide phosphorus in plasma, mg. per 100 cc.		Inorganic phosphorus in plasma, mg. per 100 cc.		Total calcium in plasma, mg. per 100 cc.		Diffusible calcium in plasma, mg. per 100 cc.		Nondiffusible calcium in plasma, mg. per 100 cc.		
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
1	This study	4	21.4-25.9	23.5	12.8-19.9	16.6	4.14- 6.36	5.38	10.18-13.46	12.50	6.75-8.71	8.11	4.28-5.05	4.81	1
2	Ashley and Guest (1934)	32-39	.....	16.2	.....	.....	.....	3.77	.....	.....	.....	.....	.....	.....	2
3	Holt (1931-32)	.....	.....	.....	.....	.....	.....	.....	.....	11.68	.....	.....	.....	.....	3
4	Gerschman (1931)	13	.....	.....	.....	.....	.....	3.30	.....	.....	.....	.....	.....	.....	4
5	Brull (1930)	5	.....	.....	.....	.....	.....	.....	10.00-14.60	12.10†	6.60-9.20	7.70†	3.00-5.40	4.40†‡	5
6	Briggs et al. (1923-24)	7	.....	.....	.....	.....	1.50- 5.50	3.00	9.80-11.60	10.60	.....	.....	.....	.....	6
7	Buell (1923)	10	.....	.....	.....	.....	3.67-10.60*	4.55*	.....	.....	.....	.....	.....	.....	7
8	Randles and Knudson (1922)	2	.....	.....	11.2-17.1*	14.3*	.....	.....	.....	.....	.....	.....	.....	.....	8

* Calculated from mg. of H_3PO_4 per 100 cc. plasma.

† Calculated average.

‡ Calculated by difference from total calcium and diffusible calcium.

SUMMARY

Normal values for the blood constituents of 50 adult dogs were obtained, and data acquired are reported in this bulletin.

The mean for the total blood volume was 76.4 cc. per kilogram of body weight; for cell volume, 36.2 cc. per kilogram of body weight; for cell volume per cent, 47.2 per cent; for red cell count, 6.83 millions per cubic millimeter; for red cell diameter, 6.98 microns; for mean corpuscular volume, 69.8 cubic microns; and for white cell count, 8.91 thousands per cubic millimeter.

The mean for oxygen-combining capacity was 17.5 volumes per cent; for total nitrogen, 3.21 grams; for nonprotein nitrogen, 0.035 gram; for protein nitrogen, 3.18 grams; for urea nitrogen, 10.47 milligrams; for amino acid nitrogen, 8.83 milligrams; and for creatinine, 1.50 milligrams per 100 cc. of whole blood.

The mean values obtained in the differential white cell count were 82.6 per cent for total neutrophiles; 11.8 per cent for total lymphocytes; 2.1 per cent for monocytes; 2.8 per cent for eosinophiles; and 0.0 per cent for basophiles.

The mean values for total nitrogen were 1.138 grams; for nonprotein nitrogen, 0.031 gram; for protein nitrogen, 1.107 grams; for fibrinogen, 0.29 gram; for albumin, 3.94 grams; for globulin, 2.44 grams; for total phosphorus, 23.5 milligrams; for lipide phosphorus, 16.6 milligrams; for inorganic phosphorus, 5.38 milligrams; for total calcium, 12.50 milligrams; for diffusible calcium, 8.11 milligrams; for nondiffusible calcium, 4.81 milligrams per 100 cc. of plasma.

No significant sex difference was observed in any of the physical or chemical measurements reported.

IV. ERYTHROCYTES AND LEUCOCYTES

ERYTHROCYTE COUNTS

As previously outlined, the present investigation consisted of both a short-term and a long-term study for each of which different groups of dogs were used as experimental subjects.

The animals were maintained on the synthetic diet and fed in amounts calculated to keep them at reasonably constant body weight. Minimum, maximum, and mean weights for each of the animals are given in table 11. Mean weights were used in calculating total blood volume, plasma volume, and cell volume, in cubic centimeters per kilogram.

Table 11. Weights of Experimental Dogs, in Kilograms

Dog No.	Minimum	Maximum	Mean
	Short-term study		
Males			
44	22.3	23.5	22.9
45	17.7	19.5	18.6
46	16.2	19.5	17.7
47	16.8	21.3	18.9
Mean	18.2	21.0	19.5
	Long-term study		
Females			
20	17.7	19.7	18.9
21	11.0	12.0	11.5
22	11.8	13.6	12.7
19	17.7	18.6	18.1
32	16.6	17.9	17.2
Mean	15.0	16.4	15.7
Males			
17	16.3	17.0	16.8
18	20.4	21.1	20.7
30	16.3	17.5	16.8
31	20.3	21.3	20.7
Mean	18.3	19.2	18.8

For the 10 experiments comprising the short-term study, the mean red cell count before bleeding was 6.86 millions per cubic millimeter; the mean percentage of the total blood volume removed through bleeding and post-bleeding sampling was 26 per cent; and on the day after hemorrhage the mean red cell count was 5.46 millions per cubic millimeter, a decrease of 1.40 millions or 20 per cent (table 12). Red cell counts dropped steadily, during the period of daily sampling, to a mean

Table 12. Values for Red Cell Counts, in Millions per Cubic Millimeter (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	7.04	5.48	5.24	5.12	4.64	4.83
	6.26	5.54	5.10	4.68	4.56	4.96
45	7.84	7.06	6.48	5.94	5.93	5.42
	8.04	7.27	6.56	6.12	5.61	5.56
46	5.52	5.53	5.00	4.84	4.61	4.72
	6.34	6.24	4.84	5.38	4.69	3.90
47	6.80	6.23	5.15	5.12	5.32	5.32
	6.54	5.24	4.96	5.32	5.18	4.90
	7.17	6.01	5.81	5.47	5.14	4.86
	7.06	6.08	5.44	5.69	4.88	4.30
Mean	6.86	6.07	5.46	5.37	5.06	4.87

level of 4.87 millions five to seven days after hemorrhage. These decreases are attributable to failure to replace the cells removed in daily sampling and to dilution resulting from increase in plasma.

In the long-term study, it was noted that in the females the mean red cell count had returned to the normal level, 6.89 millions per cubic millimeter, by the seventh week, and was somewhat above normal, 7.03 millions, by the twelfth week (table 13). In the males, the normal mean, 6.87 millions, had been regained in five weeks, and by the twelfth

Table 13. Values for Red Cell Counts, in Millions per Cubic Millimeter (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period								
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	7 wks.	12 wks.
Females										
20	6.98	5.64	7.51	4.98	5.85	7.63	5.62	5.66	6.28	7.02
21	7.08	4.80	7.10	6.64	7.36	8.15	7.44	8.67	7.90	.....
22	6.18	4.54	4.89	6.75	6.01	6.76	7.54	7.02	7.89	.....
19	7.00	5.06	4.98	5.74	5.66	6.11	6.38	5.88	6.68	8.08
32	7.21	4.66	4.76	5.09	4.82	5.64	5.20	5.99	5.80	5.98
Mean	6.89	4.94	5.85	5.84	5.94	6.86	6.43	6.64	6.91	7.03
Males										
17	7.89	5.73	6.15	7.10	6.97	7.88	7.44	8.04	7.44	7.46
18	6.99	5.24	4.54	6.34	6.20	6.48	6.48	6.58	6.45	7.68
30	6.14	5.35	5.31	5.99	5.93	6.50	7.08	6.62	.....	7.40
31	6.46	4.45	4.10	5.64	6.34	6.14	6.73	6.80	.....	6.39
Mean	6.87	5.19	5.02	6.27	6.36	6.75	6.93	7.01	6.94	7.23

week the red cell count was five per cent above normal, 7.23 millions, despite weekly sampling. This superior response on the part of the males was apparent throughout the entire investigation.

CELL SIZE

Cell diameters were not measured during the short-term study. The calculation of mean corpuscular volume resulted in a mean value of 58.4 cubic microns[1] for the last determination before hemorrhage, but it must be realized that this value does not represent the normal level based on initial measurements made on these experimental animals, since gradual decreases in mean corpuscular volume occurred throughout the study (table 14). For example, the normal value for dog 47 was 76.2 cubic microns; the last value before the first unit hemorrhage was 68.9 cubic microns. Throughout the four experiments on this animal there was a gradual decrease in mean corpuscular volume so that the last pre-bleeding value before the fourth unit hemorrhage on this animal was 45.6 cubic microns. The actual mean normal value for the four animals was 71.6 cubic microns.

Table 14. Values for Mean Corpuscular Volume, in Cubic Microns (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	54.4	60.2	59.6	55.5	56.0	57.4
	56.2	62.2	61.1	65.6	58.9	60.9
45	57.8	60.2	62.8	57.3	63.3	60.0
46	76.3	77.8	64.2	64.0	63.1	61.9
	63.0	60.5	63.4	55.6	55.6	54.1
47	68.9	63.1	70.1	66.0	61.6	66.4
	56.1	64.1	57.0	52.2	50.8	55.0
	47.0	49.2	43.7	43.9	50.7	45.5
	45.6	48.8	49.9	43.1	49.4	43.4
Mean	58.4	60.7	59.1	55.9	56.6	56.1

Determinations made 30 minutes after the start of hemorrhage showed a slight increase to 60.7 cubic microns as contrasted with the pre-bleeding mean of 58.4 cubic microns. This was followed, on the day after hemorrhage, by a mean of 59.1 cubic microns, with a decrease on the second day to 55.9 cubic microns, at which level the value remained practically unchanged during the balance of the experimental period.

[1] Based on nine experiments; one experiment, on dog No. 45, could not be included because of failure to secure a blood sample within 30 minutes of the start of bleeding.

Table 15. Values for Red Cell Diameters, in Microns (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
19	6.90	6.88	6.84	6.51	6.43	6.12	6.21	6.24	5.87
32	6.93	6.77	6.70	6.26	6.37	6.49	6.44	6.28	6.50
Mean	6.92	6.82	6.77	6.38	6.40	6.30	6.32	6.26	6.18
Males									
17	6.90	6.77	6.68	6.26	6.40	6.07	6.22	6.18	5.86
18	6.80	6.57	6.50	6.28	6.22	6.37	6.21	6.20	5.87
30	7.25	6.96	6.90	6.70	6.65	6.67	6.67	6.81	6.59
31	7.03	7.03	6.72	6.77	6.70	6.60	6.56	6.55	6.51
Mean	6.99	6.83	6.70	6.50	6.49	6.43	6.42	6.44	6.21

During the long-term study, cell-diameter measurements were made on two of the females and on the four males. The mean pre-bleeding normal cell diameter of the females was found to be 6.92 microns (table 15). Following hemorrhage, cell diameter decreased gradually until at the end of the sixth week the mean for the females was 6.26 microns, a decrease of 10 per cent. No further decrease was noted although the animals were observed for an additional six-week interval.

For the males, the mean pre-bleeding normal cell diameter was found to be 6.99 microns. As with the females, cell diameter decreased following hemorrhage, until it reached a mean of 6.44 microns at the end of six weeks, a decrease of 9 per cent, and 6.21 microns at the end of 12 weeks, a decrease of 11 per cent.

It is evident that whereas the number of red blood cells increased

Table 16. Values for Mean Corpuscular Volume, in Cubic Microns (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	59.9	57.1	46.7	53.6	52.5	43.6	53.6	52.1	46.4
21	63.3	66.4	55.8	54.5	57.9	52.8	52.8	46.2	.....
22	73.5	76.9	71.0	56.6	62.1	59.2	58.5	67.4	.....
19	81.4	69.6	78.9	68.1	.....	59.9	58.3	62.4	43.4
32	71.1	72.5	65.3	67.5	69.7	64.2	67.1	60.3	62.7
Mean	69.8	68.5	63.5	60.1	60.5	55.9	58.1	57.7	50.9
Males									
17	64.9	71.2	57.6	50.0	60.4	49.1	52.4	46.5	46.9
18	66.0	58.4	73.3	56.0	54.5	56.5	55.1	51.8	46.6
30	65.6	58.3	62.5	62.8	67.4	65.5	64.1	65.0	62.6
31	70.6	73.9	.....	69.0	64.5	71.8	68.8	63.5	72.1
Mean	66.8	65.4	64.5	59.4	61.7	60.7	60.1	56.7	57.0

during the long-term study to a level considerably above normal, cell diameter decreased progressively, the females showing a more rapid decline than the males.

The pre-bleeding normal mean corpuscular volume for the five females used in this long-term study was 69.8 cubic microns (table 16). Following hemorrhage, there was a steady reduction in mean corpuscular volume until it reached a mean level of 57.7 cubic microns at the end of the sixth week, a decrease of 17 per cent. For the three females observed for 12 weeks, mean corpuscular volume at the end of that period was 50.9 cubic microns, contrasted with the mean level of 58.3 cubic microns found for the same animals at the end of the sixth post-bleeding week.

For the males, the pre-bleeding normal mean corpuscular volume was 66.8 cubic microns. By the end of the sixth week following hemorrhage, this level had been reduced to 56.7 cubic microns, a decrease of

Table 17. Effect of Repeated Hemorrhage on Values for Mean Corpuscular Volume, in Cubic Microns, and Red Cell Diameter, in Microns

Dog No.	Mean corpuscular volume		Red cell diameters	
	Normal	After repeated hemorrhages	Normal	After repeated hemorrhages
Males				
36	76.6	49.5	7.21	5.29
37	72.1	52.5	6.95	5.59
38	75.0	56.4	7.00	5.28
Mean	74.6	52.8	7.05	5.39

15 per cent, contrasted with a 9 per cent decrease in cell diameter. During the second six-week portion of the post-bleeding period, no further decrease was noted, the value observed at the end of the twelfth post-bleeding week being 57.0 cubic microns. On a percentage basis, therefore, there was a greater decrease in mean corpuscular volume than in cell diameter. This was further substantiated by the results of the study on the three male animals that had been subjected to four or five bleedings each and then observed for approximately four months after bleeding (table 17). One dog, number 36, displayed a 35 per cent reduction in mean corpuscular volume, from a normal of 76.6 cubic microns to 49.5 cubic microns, and a 26 per cent reduction in cell diameter, from a normal of 7.21 microns to 5.29 microns. A second animal, number 37, showed a 27 per cent reduction in mean corpuscular volume, from a normal of 72.1 cubic microns to 52.5 cubic microns, with a 20 per cent reduction in cell diameter, from a normal of 6.95 microns to 5.59 microns. The third dog, number 38, showed a 25 per cent reduction in mean corpuscular volume, from a normal of 75.0 cubic microns to 56.4 cubic microns, with a 24 per cent reduction in cell

diameter, from a normal of 7.00 microns to 5.28 microns. The mean decrease in mean corpuscular volume was 29 per cent, and the mean decrease in cell diameter was 24 per cent.

TOTAL CELL VOLUME

A mean pre-bleeding total cell volume of 574.7 cc. was observed for the four animals used in the short-term study (table 18). A mean cell volume of 149.4 cc. was removed through bleeding and post-bleeding sampling, which left a predicted mean cell volume of 425.3 cubic centimeters. The actual mean cell volume, observed within 30 minutes of the start of bleeding, was 447.3 cc., a difference of 22.0 cubic centimeters. Sampling for analysis resulted in the removal of an additional volume of 25.4 cc. of cells before blood-volume determinations were made 24 hours after hemorrhage. Deducting this quantity from the actual value for mean cell volume after bleeding, 447.3 cc., would leave a net predicted mean cell volume of 421.9 cubic centimeters. Actually, the mean cell volume observed 24 hours after hemorrhage was 415.4 cubic centimeters. Therefore, the observed values approximated the predicted values to a surprising degree.

Table 18. Actual and Predicted Total Cell Volume, in Cubic Centimeters, 30 Minutes and 24 Hours After Bleeding
(Short-term study)

Dog No.	Last pre-bleeding	Cell volume removed in bleeding and sampling	Within 30 minutes of start of bleeding		Cell volume removed in sampling	24 hours after bleeding	
			Predicted cell vol.	Actual cell vol.		Predicted cell vol.	Actual cell vol.
Males							
44	669.2	231.5	437.7	472.9	26.9	446.0	484.1
	603.7	129.0	474.7	448.6	24.2	424.4	470.5
45	672.3	147.1	525.2	475.2	29.0	446.2	564.2
46	605.0	141.8	463.2	562.1	23.3	538.8	437.2
	569.9	181.7	388.2	522.2	25.8	496.4	353.7
47	649.4	136.6	512.8	495.1	24.8	470.3	452.2
	537.1	139.0	398.1	414.2	23.3	390.9	338.9
	452.6	120.6	332.0	311.0	26.6	284.4	279.6
	413.0	117.3	295.7	324.4	24.8	299.6	358.2
Mean	574.7	149.4	425.3	447.3	25.4	421.9	415.4

In terms of body weight, the pre-bleeding mean cell volume of the animals used for the short-term study was 30.5 cc. per kilogram (table 19). Again it should be pointed out that this value does not represent the normal mean cell volume for these animals, since gradual decreases in this factor occurred throughout the study. Within 30 minutes after the start of hemorrhage, the observed mean level was 23.2 cc. per kilogram, and on the day following bleeding, a value of 21.8 cc. per kilogram

Table 19. Values for Cell Volume, in Cubic Centimeters per Kilogram (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	29.2	20.6	21.1	18.6	16.3	16.8
	26.4	19.6	20.5	20.1	17.1	19.4
45	38.6	.....	25.7	25.9	23.5	22.7
	36.0	25.6	30.2	22.8	23.6	21.3
46	34.2	31.8	24.8	22.4	22.2	21.8
	32.2	29.5	20.0	20.9	17.9	15.3
47	34.4	26.2	23.9	19.2	18.3	23.0
	28.4	21.9	17.9	18.7	17.7	18.3
	24.0	16.5	14.8	14.5	15.3	12.7
	21.8	17.2	19.0	15.6	16.0	15.4
Mean	30.5	23.2	21.8	19.9	18.8	18.7

was obtained. On succeeding days further small decreases followed the removal of cells through sampling for analysis, resulting in a mean value of 18.7 cc. per kilogram five to seven days after bleeding.

In the long-term study, the mean normal cell volume for the five female dogs was 32.1 cc. per kilogram (table 20). One week after the removal of approximately one half the total blood volume, the mean cell volume was 20.7 cc. per kilogram. Recovery throughout the post-bleeding period was slow, attaining a mean cell volume of 22.4 cc. per kilogram at the end of the sixth week. For the three dogs that were observed for 12 weeks, a mean cell volume of 19.8 cc. per kilogram was noted at the end of the twelfth week, as compared with a mean of 19.3

Table 20. Values for Cell Volume, in Cubic Centimeters per Kilogram (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	27.2	19.3	19.7	14.6	20.4	19.8	17.8	18.9	21.5
21	29.5	22.1	25.2	24.0	23.1	26.8	23.9	25.4	.....
22	28.1	20.8	17.6	22.3	21.9	22.8	26.9	28.7	.....
19	41.3	22.7	22.1	22.0	.....	21.8	19.6	18.6	16.5
32	34.2	19.1	18.7	19.5	19.0	21.3	19.9	20.4	21.5
Mean	32.1	20.8	20.7	20.5	21.1	22.5	21.6	22.4	19.8
Males									
17	36.7	24.4	22.6	22.9	29.0	23.9	22.8	24.4	20.6
18	26.9	16.4	16.6	17.8	19.2	22.1	19.3	18.1	20.1
30	29.4	22.5	24.3	29.3	31.6	32.1	38.8	33.5	34.6
31	30.4	23.6	24.6	27.6	28.9	31.7	34.9	30.7	32.5
Mean	30.8	21.7	22.0	24.4	27.2	27.4	28.9	26.7	27.0

cc. at the end of the sixth week. It is evident that the volume of cells removed in weekly sampling tended to offset any cell volume regeneration.

For the male animals, the pre-bleeding normal cell volume was 30.8 cc. per kilogram. At the end of the first week following hemorrhage, the volume was 22.0 cc. per kilogram. During the post-bleeding period, a gradual increase in cell volume per unit of body weight was noted in these animals. A mean level of 26.7 cc. was observed at the end of the sixth week and a mean level of 27.0 cc. per kilogram was noted at the end of the twelfth week. Apparently, therefore, the male dogs were capable of a somewhat more satisfactory response than the females. However, since recovery was not complete, even after 12 weeks, it would appear that in the male dogs also weekly sampling played an important part in limiting the degree of recovery.

In adjusting the food level to maintain a fairly constant body weight, some dogs received more food per kilogram than others. Accordingly, to ascertain whether food intake was a factor in the superior performance of the male animals, the amount of food consumed in the first six weeks of the post-bleeding period was calculated in terms of body weight. For the males, it was found to be 471.9 g. per kilogram; for the females, 539.0 g. (table 21). The more satisfactory response of the males, therefore, cannot be attributed to a greater food intake per kilogram of body weight.

Table 21. Amount of Synthetic Ration Consumed, in Grams per Kilogram (Six-Week Period) (Long-term study)

Females		Males	
Dog. No.	Amount consumed	Dog. No.	Amount consumed
20	591.0	17	499.1
21	669.5	18	367.1
22	564.9	30	575.8
19	419.8	31	445.6
32	450.0		
Mean	539.0	Mean	471.9

Since the recovery of the male dogs was appreciably better than that of the female dogs during the first six weeks of the post-bleeding period, and since, too, the mean weight of the females was somewhat less than that of the males (15.7 kg. and 18.8 kg., respectively), it seemed advisable to ascertain whether the slower recovery of the females could be ascribed to the fact that a larger percentage of total cell volume was removed from the females than from the males in the course of bleeding and weekly sampling. Accordingly, the total volume of cells removed during bleeding and weekly sampling in the first six weeks of the post-bleeding period was calculated for both males and females and expressed

Table 22. Percentage of Total Cell Volume Removed Through Bleeding and Weekly Sampling (Six-Week Period) (Long-term study)

Females		Males	
Dog No.	Percentage	Dog No.	Percentage
20	61.9	17	79.5
21	83.6	18	68.1
22	86.6	30	75.3
19	60.1	31	68.2
32	62.7		
Mean	71.0	Mean	72.8

in terms of percentage of original total cell volume. For the males, the mean was found to be 72.8 per cent; for the females, 71.0 per cent (table 22). From these figures, it is evident that the females' retarded recovery cannot be attributed to the loss of a relatively greater quantity of blood cells.

The volume of cells regenerated during the first six weeks of the post-bleeding period, per kilogram of body weight, was also calculated for each dog, resulting in a mean cell volume regeneration of 13.0 cc. per kilogram for the females, as against a mean of 18.8 cc. per kilogram for the males (table 23).

There was some variation from animal to animal in the percentage of total cell volume removed (table 22). Moreover, wide variations in the volume of cells regenerated were evident within the groups (table 23). Therefore, as a search for an explanation of the observed differences in response, a correlation was run between the percentage of cells removed and cell volume regenerated during the first six weeks of the post-bleeding period. Although the correlation coefficient obtained with this series, +.8128, was not significant, owing to the limited number of dogs involved, a similar calculation, based on data secured in this investigation plus unpublished data covering other experiments per-

Table 23. Cell Volume Regenerated, in Cubic Centimeters per Kilogram (Six-Week Period) (Long-term study)

Females		Males	
Dog No.	Cell volume regenerated	Dog No.	Cell volume regenerated
20	8.7	17	17.8
21	21.0	18	9.6
22	25.4	30	26.5
19	2.2	31	21.5
32	7.8		
Mean	13.0	Mean	18.8

formed in this laboratory on an additional group of dogs, resulted in a significant positive correlation, +.6152, indicating that one condition that influenced cell volume regeneration was the degree of physiological stress induced by bleeding.

The ranges observed in cell volume regeneration, 2.2 to 25.4 cc. per kilogram for the females, and 9.6 to 26.5 cc. per kilogram for the males, suggested that another factor, in addition to physiological stress, was decidedly important in controlling the volume of cells regenerated following hemorrhage. The most obvious explanation was a reserve of blood-building materials in the animal body at the time of hemorrhage. Assuming that such a reserve existed, and in order to discover the extent to which it was a determining influence, the first two or three weeks following the initial bleeding were set up as a "depletion period" during which the reserves in the animal body might be utilized for the regeneration of cells. This interval apparently allowed for depletion of any

Table 24. Cell Volume Regeneration in Cubic Centimeters per Kilogram per Week (Long-term study)

Dog No.	Depletion period (2 to 3 weeks)	Post-depletion period (4 to 10 weeks)
Females		
20	1.19	1.58
21	2.45	1.65
22	2.07	2.32
19	1.08	0.37
32	2.49	0.99
Mean	1.86	1.38
Males		
17	1.56	1.29
18	2.66	1.13
30	6.26	2.21
31	5.73	1.64
Mean	4.05	1.57

reserves since cell volume regeneration during the remainder of the post-bleeding period continued at a reduced but fairly constant rate. The weekly volume of cells regenerated during the depletion period was calculated for each animal on the basis of body weight, as was the volume of cells regenerated during the "post-depletion period," and these data are presented in table 24.

It was evident during the depletion period that the males were capable of appreciably better cell volume regeneration than the females. Although the marked difference between the means for the males and females (4.05 cc. per kilogram per week for the males as against 1.86 cc. for the females) was attributable in a large measure to the outstanding performance of two of the males, nevertheless three of the male animals produced higher values than any of the females, and the male

that showed the least regeneration was definitely higher than the lowest females. When Student's "t" test was applied to these data, the difference in the performance of the males and females just fell short of being significant ($P = <0.086$). During the post-depletion period, however, the males' mean cell volume regeneration, 1.57 cc. per kilogram per week, was only slightly better than that of the females, 1.38 cc. per kilogram per week.

Table 24 also shows individual differences within both groups of animals in their ability to regenerate cells. For the females, cell volume regeneration ranged, during the depletion period, from 1.08 to 2.49 cc. per kilogram per week, whereas for the males it ranged from 1.56 to 6.26 cc. per kilogram per week. During the post-depletion period, the range for the females was from 0.37 to 2.32 cc., and for the males, from 1.13 to 2.21 cc. per kilogram per week.

It seemed possible that the amount of food consumed might offer an explanation for these individual differences in the rate of cell volume regeneration during the post-depletion period. It will be recalled that in adjusting the food level to maintain a fairly constant body weight, some animals received more food per kilogram than others. Accordingly, to ascertain to what extent food intake might be a factor in the higher cell volume regeneration level of some animals, the amount of food consumed per kilogram per week was calculated for each of the animals and a correlation run between the amount of food consumed and the volume of cells regenerated per kilogram per week. The correlation coefficient obtained was significant, +.7873, and demonstrated that cell volume regeneration was at least in part controlled by the amount of food intake. It should be reiterated, however, that this does not explain the difference between the males and females since the females actually consumed more food per kilogram than did the males.

With the thought that the normal cell volume, in cubic centimeters per kilogram, might be one criterion of the blood-building reserves in the animal body, another correlation was run between normal cell volume and the volume of cells regenerated in cubic centimeters per kilogram during the first six weeks of the post-bleeding period. The result was an insignificant correlation coefficient, indicating that no relationship existed between these two factors. It is apparent that the normal cell volume is no indication of the blood-building reserves in the animal body, or at least it is no index of the hemopoietic response which will follow the loss of a considerable amount of blood.

CELL VOLUME PER CENT

The rapidity with which interstitial fluid is drawn into the blood stream in an effort to restore blood volume following the loss of a significant amount of blood is of vital interest.

Table 25. Values for Cell Volume Per Cent
(Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	38.6	33.0	31.2	28.4	26.0	26.8
	35.2	34.5	31.2	30.7	26.9	28.7
45	46.5	43.8	41.2	35.1	35.5	48.5
46	42.1	43.0	32.1	31.0	29.1	30.7
	39.9	37.8	30.7	29.9	26.1	23.0
47	46.8	39.3	36.1	33.8	32.8	29.1
	38.3	33.6	28.3	27.8	26.3	26.9
	33.7	29.6	25.4	24.8	26.0	22.1
	32.2	29.7	27.1	24.5	24.1	23.5
Mean	39.2	36.0	31.4	29.5	28.1	28.7

Table 26. Values for Cell Volume Per Cent
(Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	39.7	32.2	35.1	26.7	30.7	33.3	30.1	29.5	32.6
21	44.8	31.9	39.6	36.2	42.6	43.0	39.3	40.1	.....
22	45.4	34.9	34.7	38.2	37.3	40.0	44.1	47.3	.....
19	58.8	35.2	39.3	39.1	.....	36.6	37.2	36.7	35.1
32	49.6	33.8	31.1	33.8	33.6	36.2	34.9	36.1	37.5
Mean	47.6	33.6	35.9	34.8	36.0	37.8	37.1	37.9	35.1
Males									
17	46.8	40.8	35.4	35.5	42.1	38.7	39.0	37.4	35.0
18	46.1	30.6	33.3	35.5	33.8	36.6	35.7	34.1	35.8
30	39.0	31.2	33.2	37.6	40.0	42.6	45.4	43.0	46.3
31	42.6	32.9	35.5	38.9	40.9	44.1	46.3	43.2	46.1
Mean	43.6	33.9	34.4	36.9	39.2	40.5	41.6	39.4	40.8

In the short-term study, it was observed that the mean pre-bleeding cell volume per cent in nine[2] experiments was 39.2 per cent (table 25). Analyses made within 30 minutes of the start of hemorrhage returned a mean cell volume per cent of 36.0, indicating that within this relatively short period an appreciable amount of dilution had occurred. Complete adjustment followed within the next 24 hours. On the day after bleeding, the observed mean cell volume per cent was 31.4 per cent; on the second day, 29.5 per cent; on the third day, 28.1 per cent. These later decreases resulted from the loss of cells in daily sampling and were un-

[2] One experiment, on dog number 45, was omitted since no value was obtained within 30 minutes of the start of hemorrhage.

doubtedly also influenced by the alterations noted in mean corpuscular volume.

In the long-term study, the mean pre-bleeding cell volume per cent for the females was 47.6 per cent (table 26). One week after hemorrhage the mean was 35.9 per cent, and at the end of six weeks, 37.9 per cent, showing little recovery. For the three females observed for 12 weeks, the mean at the end of six weeks was 34.1 per cent; at the end of 12 weeks, 35.1 per cent, indicating little improvement in the second six-week interval.

For the males, the mean pre-bleeding normal cell volume per cent was 43.6 per cent. At the end of the first post-bleeding week the mean was 34.4 per cent; at the end of six weeks, 39.4 per cent; and at the end of 12 weeks, 40.8 per cent. In cell volume per cent, as in cell count and cell volume regeneration, the males responded more satisfactorily than the females, but, just as with the females, they displayed little improvement during the second six-week portion of the post-bleeding period.

INTERRELATIONSHIPS BETWEEN COUNT, SIZE, AND VOLUME OF CELLS

In the long-term study, the coefficient of correlation obtained between cell count and cell diameter (−.7745) was significant, indicating a close relationship between cell diameter and the number of cells regenerated in hemorrhagic anemia.

Between cell diameter and mean corpuscular volume another significant coefficient of correlation was obtained (+.4411). Alterations in the other dimensions of the cell, which are not directly proportional to the change in diameter, probably explain why this correlation was no higher. Although in certain types of anemia there have been observed cells of unusual shapes which would result in an altered relationship between the volume of the erythrocyte and its diameter, cells abnormal in contour were not found, even in severely anemic dogs, in the present study. The correlation coefficient obtained between cell count and cell volume in cubic centimeters per kilogram (+.3524), while significant, indicates that cell count is not an entirely satisfactory measure of total cell volume. No relationship was observed between cell diameter and cell volume in cubic centimeters per kilogram.

OXYGEN-COMBINING CAPACITY

The oxygen-combining capacity of the blood is usually accepted as the most significant measure of the degree of anemia.

In the short-term study, the mean pre-bleeding oxygen capacity for the 10 experiments was found to be 15.0 volumes per cent (table 27). The decrease observed within 30 minutes after the start of hemorrhage, to 14.4 volumes per cent, was not so great as might have been anticipated

Table 27. Values for Oxygen-combining Capacity, in Volumes Per Cent (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	15.6	13.9	11.9	10.9	10.8	10.7
	14.0	14.8	13.2	12.2	10.7	11.3
45	18.8	18.5	14.7	16.6	14.7	14.6
	16.8	17.7	14.0	12.6	13.6	11.6
46	17.3	16.0	13.4	13.0	12.5	12.3
	15.0	13.6	11.3	13.1	10.6	9.1
47	14.0	11.8	12.3	13.4	12.6	11.2
	13.3	13.4	11.0	11.3	11.1	6.5
	13.0	11.5	10.2	10.0	8.0	8.1
	12.4	12.3	9.6	10.1	9.1	8.3
Mean	15.0	14.4	12.2	12.3	11.4	10.4

from the change in cell volume per cent (39.2 to 36.0 per cent). Since the sample used for determining oxygen capacity was always secured before the determination of cell volume per cent, it is possible that the time elapsing between the securing of these two samples might have accounted for the lack of agreement. Unquestionably this was a period during which there was an exceedingly rapid infiltration of cellular fluid into the blood stream.

On the first day after hemorrhage, the mean oxygen capacity observed was 12.2 volumes per cent. It is interesting to note the close agreement between the decrease in cell volume per cent (19.9 per cent) and the decrease in oxygen capacity (18.7 per cent), although the agreement between these two factors was not quite so close on the second and third days after hemorrhage. For example, by the third day after bleeding, the observed decrease in cell volume per cent was 28.3 per cent as contrasted with a decrease in oxygen capacity of 24.0 per cent. Nevertheless, it would seem that these values are as close as could be expected and are not indicative of alterations in the hemoglobin concentration in the cell.

In the long-term study, the pre-bleeding normal oxygen capacity for the females was 18.0 volumes per cent (table 28). At the end of the first week, following loss of approximately one half of the determined blood volume, the mean oxygen capacity was 12.6 volumes per cent. A further slight decrease occurred during the first six weeks of the post-bleeding period, the oxygen capacity at the end of the sixth week being 11.4 volumes per cent. For the three females observed for 12 weeks, the mean at the end of the twelfth week was 11.8 volumes per cent as compared with 11.7 volumes per cent for these same dogs at the end of the sixth week. Evidently the weekly loss of from 30 to 50 cc. of blood through sampling offset any recovery that was made.

Table 28. Values for Oxygen-combining Capacity, in Volumes Per Cent (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	14.2	9.8	12.7	11.5	9.1	9.5	7.1	10.7	10.7
21	14.7	11.6	13.5	11.3	12.2	12.0	11.7	10.8	.....
22	18.5	10.4	9.2	9.9	8.8	10.7	11.6	11.0	.....
19	22.8	16.0	16.4	14.5	15.0	15.2	13.8	12.2	10.8
32	19.7	11.8	11.1	11.0	11.1	11.9	12.3	12.3	13.8
Mean	18.0	11.9	12.6	11.6	11.2	11.9	11.3	11.4	11.8
Males									
17	18.9	13.9	13.5	14.6	13.5	15.1	14.7	14.0	11.3
18	17.3	12.7	.....	10.4	11.0	10.5	11.6	11.4	10.5
30	17.3	10.5	13.0	13.2	12.9	15.6	15.7	14.8	16.9
31	18.7	11.9	11.4	12.6	13.4	14.0	16.9	15.9	15.2
Mean	18.0	12.2	12.6	12.7	12.7	13.8	14.7	14.0	13.5

For the males, the pre-bleeding oxygen capacity was 18.0 volumes per cent; at the end of the first week of the post-bleeding period it was 12.6 volumes per cent. Attention is called to the fact that these values are identical with those reported for the females. The means for oxygen capacity for the males, observed at the end of six weeks and 12 weeks, were 14.0 and 13.5 volumes per cent, respectively. Although these values were somewhat higher than those reported for the females, they were still considerably below the pre-bleeding mean for these animals.

Table 29. Oxygen-combining Capacity—Comparison of Normal Levels with Levels Observed Six Weeks After Hemorrhage (Long-term study)

Dog No.	Pre-bleeding normal in volumes per cent	At end of sixth post-bleeding week in volumes per cent	Differences in volumes per cent
Females			
20	14.2	10.7	— 3.5
21	14.7	10.8	— 3.9
22	18.5	11.0	— 7.5
19	22.8	12.2	—10.6
32	19.7	12.3	— 7.4
Mean	18.0	11.4	— 6.6
Males			
17	18.9	14.0	— 4.9
18	17.3	11.4	— 5.9
30	17.3	14.7	— 2.6
31	18.7	15.9	— 2.8
Mean	18.0	14.0	— 4.0

Since there were such marked individual differences in the degree of recovery shown by the animals, table 29 was prepared to show the normal oxygen capacity in volumes per cent, the level at the end of the first six weeks of the post-bleeding period, and the difference between the two levels. The smaller the difference, the more closely did the animal approach his pre-bleeding level and attain complete recovery.

An effort was made to ascertain whether the degree of recovery as measured by these differences was related to the normal oxygen capacity of the blood by running a correlation between the two sets of values. The correlation coefficient obtained, +.7358, was not significant. Again, based on data secured in this investigation plus unpublished data covering other experiments performed in this laboratory on an additional group of dogs, a significant correlation coefficient, +.5458, was obtained, indicating that the animals with the highest normal levels were the furthest from their own normal levels at the end of the post-bleeding period.

It is generally assumed that 1.34 cc. of oxygen is equivalent to 1.0 g. of hemoglobin. Accordingly, this value together with the volume of cells was used as the basis for calculating hemoglobin regeneration

Table 30. Hemoglobin Regeneration in Grams per Kilogram per Week (Long-term study)

Dog No.	Depletion period (2 to 3 weeks)	Post-depletion period (4 to 10 weeks)
Females		
20	0.49	0.20
21	0.14	0.39
22	0.11	0.46
19	−0.11	0.05
32	0.38	0.42
Mean	0.20	0.30
Males		
17	0.49	0.13
18	0.55	0.25
30	1.81	0.57
31	1.04	0.65
Mean	0.97	0.40

during the post-bleeding period. The first two or three weeks following the initial bleeding were set up as a depletion period, as in the case of cell volume regeneration. Hemoglobin regeneration, per kilogram per week, was then calculated for the depletion period and also for the remainder of the post-bleeding period. The results are given in table 30, which demonstrates that during the depletion period the males showed a considerably greater capacity for regenerating hemoglobin than they did during the post-depletion period, the mean for the depletion

period being 0.97 g., and for the post-depletion period, 0.40 g. per kilogram per week. This difference failed to be statistically significant when tested by means of the standard error of the difference ($P = 0.080$). The females, on the other hand, failed to show this greater capacity for regeneration during the depletion period, the mean value observed, 0.20 g., being in fact slightly below the mean noted for the post-depletion period, 0.30 g. per kilogram per week, from which it is apparent that any reserve the females may have had must have been exhausted, perhaps even before bleeding, possibly in the sampling necessary for the establishment of the normal levels.

In the depletion period, the male animals were capable of regenerating hemoglobin more rapidly than the females, as evidenced by Student's "t" test ($P = 0.035$). During the post-depletion period, however, although the males continued to display some superiority over the females in the regeneration of hemoglobin, the difference between the two groups was not very large.

As with cell volume regeneration, wide individual differences were apparent in the various animals' capacity for hemoglobin regeneration. For the females the range during the depletion period was from -0.11 to $+0.49$ g. per kilogram per week, and for the males, from 0.49 to 1.81 g. per kilogram per week. During the post-depletion period the observed range for the females was from 0.05 to 0.46 g. per kilogram per week, and for the males, from 0.13 to 0.65 g. per kilogram per week.

To test whether food intake influenced the degree of hemoglobin regeneration during the post-depletion period, a correlation was run between food intake in grams per kilogram per week and hemoglobin regeneration in grams per kilogram per week. The correlation coefficient obtained, $+.4192$, was not significant, indicating the hemoglobin regeneration was not related to the level of food intake, a result contrary to that obtained for cell volume regeneration.

LEUCOCYTES

The immediate effect of hemorrhage, as shown in the short-term study, was to decrease slightly the number of leucocytes per cubic millimeter of blood, from a normal of 7.50 thousands to 7.05 thousands (table 31). No doubt this decrease, as with the red blood cells, was due to dilution of the blood with interstitial fluid. The mean white cell counts on succeeding days were somewhat above the pre-bleeding level, being 8.13, 8.47, and 7.89 thousands, on the first, second, and third days, respectively, suggesting a mild leucocytosis as a result of hemorrhage. However, calculation of the standard error of the difference showed that the observed increase was not significant.

In the long-term study, there was also some increase in leucocytes, which again proved to be insignificant. For the females, at the end of the first week, the mean count was 10.30 thousands per cubic millimeter

Table 31. Values for Leucocyte Counts, in Thousands per Cubic Millimeter (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	7.20	5.08	6.24	6.64	6.01	6.54
	8.89	5.90	6.99	7.45	6.21	6.21
45	4.75	3.65	5.45	5.02	6.89	5.52
	5.58	5.02	6.08	7.34	6.86	6.55
46	9.44	14.04	9.54	9.39	8.34	7.78
	6.78	6.06	8.72	9.59	7.59	7.79
47	6.34	7.46	8.86	7.70	8.21	7.89
	8.88	8.62	10.34	12.48	10.99	10.44
	10.06	7.38	11.02	10.02	9.38	17.82
	7.10	7.32	8.10	9.09	8.40	8.34
Mean	7.50	7.05	8.13	8.47	7.89	8.49

of blood, contrasted with a pre-bleeding mean of 5.85 thousands (table 32). By the end of the sixth week, the mean count was still slightly above normal (6.57 thousands per cubic millimeter), but by the end of the twelfth week, the count (based on three dogs) had returned to normal. For the males, the pre-bleeding mean was 7.41 thousands per cubic millimeter. By the end of the sixth and twelfth weeks, the mean counts were 7.71 and 7.56 thousands, respectively. Values slightly above the mean normal count were observed one to two days after bleeding (8.59 thousands) and again at the end of the second week

Table 32. Values for Leucocyte Counts, in Thousands per Cubic Millimeter (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	9.55	5.40	8.95	7.10	6.55	7.20	9.80	5.30	6.10
21	3.25	7.00	13.45	7.15	4.75	5.40	5.15	10.25	.....
22	4.15	10.20	15.60	13.45	9.25	7.30	8.80	4.50	.....
19	4.55	4.15	5.55	4.50	5.15	4.45	3.60	3.90	3.35
32	7.75	8.40	7.95	7.70	9.00	6.95	7.45	8.90	6.75
Mean	5.85	7.03	10.30	7.98	6.94	6.26	6.96	6.57	5.40
Males									
17	5.30	9.45	9.45	9.55	10.10	5.45	7.15	12.00	9.30
18	5.10	7.55	6.80	7.20	8.70	5.80	6.50	4.25	5.40
30	8.80	8.75	5.25	17.00	8.80	5.70	7.85	6.50	6.55
31	10.45	8.60	8.25	8.40	7.50	8.65	8.15	8.10	9.00
Mean	7.41	8.59	7.44	10.54	8.77	6.40	7.41	7.71	7.56

(10.54 thousands) and third week (8.77 thousands), with essentially normal values for the other experimental weeks. The mean for the second week (10.54 thousands) was influenced markedly by the high value observed for dog number 30, although two of the other males were also somewhat above their normal levels at that time.

It seemed possible that more pronounced changes in the total or differential leucocyte count might be found in the dogs subjected to repeated bleedings (third study). These three animals were bled four or five times each, but no increase in the total leucocyte count was noted as a result of repeated hemorrhage (table 33).

Table 33. Values for Leucocyte Counts, in Thousands per Cubic Millimeter (Dogs subjected to repeated bleedings)

Dog No.	Pre-bleeding	Post-bleeding
Males		
36	13.2	12.0
	12.0	9.9
	10.0	14.5
	11.5	10.2
	12.6	13.7
37	10.0	11.4
	11.4	9.1
	9.1	9.6
	7.3	8.0
38	8.7	11.4
	11.4	11.3
	11.3	13.7
	11.5	10.2
	14.3	13.6
Mean	11.0	11.3

On the basis of the differential leucocyte count there would appear to be a small increase in the young band-form neutrophiles as a result of hemorrhage and a corresponding decrease in total lymphocytes involving both the small and large forms. The percentage of the other forms of leucocytes did not appear to be noticeably altered (table 34).

DISCUSSION

Anemia of hemorrhagic origin is found often enough in human subjects to make a study of the complete blood picture a matter of considerable importance.

The purpose of this investigation was to secure a more thorough understanding than has been general up to the present time of conditions that exist in the blood of a subject suffering from hemorrhagic anemia. Accordingly, all factors that seemed to offer a clue to the difficulties

Table 34. Differential Leucocyte Counts (Dogs subjected to repeated bleedings)

Dog No.	Percentage of neutrophiles						Percentage of lymphocytes				Percentage of monocytes		Percentage of eosinophiles		Percentage of basophiles	
	Immature		Young band forms		Mature (segmented)		Small		Large							
	Before bleeding	After bleeding	Before bleeding	After bleeding	Before bleeding	After bleeding	Before bleeding	After bleeding	Before bleeding	After bleeding	Before bleeding	After bleeding	Before bleeding	After bleeding	Before bleeding	After bleeding
Males																
36	0.5	0.0	17.5	18.5	53.5	58.5	13.5	12.5	4.0	5.0	2.5	1.5	8.5	4.0	0.0	0.0
	0.0	4.5	25.5	25.0	53.0	40.0	14.5	20.5	2.5	1.0	1.5	1.5	2.5	5.0	0.5	2.5
	1.0	0.5	11.0	12.5	65.5	67.0	11.5	8.5	1.0	0.5	1.5	1.0	8.5	10.0	0.0	0.0
37	2.0	0.5	15.5	22.0	54.0	56.5	23.0	16.0	4.0	2.5	1.5	2.5	0.0	0.0	0.0	0.0
	0.5	3.0	8.5	13.5	67.5	63.5	14.0	12.0	2.0	0.5	2.5	1.0	5.0	6.5	0.0	0.0
38	0.0	0.5	8.5	13.5	65.0	68.5	16.5	11.5	5.0	3.0	5.0	2.5	0.0	0.5	0.0	0.0
	0.5	1.5	13.5	17.5	68.5	68.5	11.5	7.5	3.0	1.5	2.5	1.0	0.5	2.5	0.0	0.0
	0.5	0.0	14.0	20.0	69.5	58.0	8.5	15.0	0.0	0.0	1.0	0.5	6.5	6.0	0.0	0.5
	0.0	0.5	9.5	7.0	65.5	72.5	21.5	7.5	1.0	0.0	0.5	0.5	2.0	12.0	0.0	0.0
Mean	0.5	1.2	13.7	16.6	62.4	61.4	14.9	12.3	2.5	1.5	2.0	1.3	3.7	5.2	0.0	0.3

involved in recovery following loss of appreciable amounts of blood have been studied simultaneously and will be discussed in the following paragraphs.

Erythrocyte Counts

It is generally assumed that the animal body has reserve stores of cells, said to be located largely in the spleen, upon which it can draw in an emergency. From the data obtained through the present research, however, it would appear that either such stores were not available in these experimental animals, or, although this seems highly improbable, the losses of blood to which they were subjected were not sufficiently drastic to release these stores. At the same time, it was demonstrated that animals do have varying quantities of cell-building materials which are used during the early stages of recovery for blood regeneration.

It is evident from the results of this investigation that red cell counts, oxygen-combining capacity determinations, and cell volume per cent readings alone will not indicate the severity of the anemia that follows acute loss of blood. Nevertheless, these factors must be considered in conjunction with total blood volume determinations in order to obtain a true picture of a subject's condition. This is demonstrated by results obtained in the short-term study. Had the total blood volume been restored to normal entirely through dilution with interstitial fluid, the decrease in red cell count 24 hours after hemorrhage would have been 26 per cent. However, only about two thirds of the loss in blood volume was restored, and the reduction in red cell count was only 20 per cent. Assuming that the restoration of blood volume was wholly in the plasma fraction, this degree of dilution would have resulted in a decrease in red cell count to 5.56 millions, whereas the observed value was 5.46 millions. With these two values in such close agreement, it is apparent that the recovery in total blood volume must have been due entirely to an increase in plasma and not to a replacement of any portion of the lost cells.

In the long-term study, red cell counts returned to normal five to seven weeks after hemorrhage. In the males the normal level had been regained in five weeks; in the females, not until the seventh week. These data are of greater significance in conjunction with those on cell size and total cell volume. Therefore, further discussion will be reserved for inclusion with those factors.

Cell Size

In recent years cell size has been receiving increasing emphasis as a diagnostic measure, although as early as 1924-25 Haden stated that the extent of the decrease in cell size in chronic hemorrhagic anemia was probably the best measure of the drain on the bone marrow and

was accordingly of definite prognostic value. Witts (1931) pointed out that in human subjects chronic microcytic anemia is essentially a disease of the female sex and of the reproductive period and its occurrence in men is an indication of blood loss through accidents, gastric hemorrhage, and the like. Wright (1933), Barer, Fowler, and Baldridge (1934-35), and Fowler and Barer (1937) postulated that certain cases of idiopathic microcytic or hypochromic anemia might be associated with relatively large menstrual losses, while Wintrobe (1934) suggested a classification of anemias on the basis of the size and hemoglobin content of the erythrocytes.

In the short-term study, the slight increase in mean corpuscular volume noted within 30 minutes of the start of bleeding may have been due to hyperemia resulting from a lowered colloid osmotic pressure of the plasma brought about by the observed decrease in both plasma protein and lipide phosphorus, or it may have been the result of alterations in the hydrogen-ion concentration of the plasma. Bennett (1926) demonstrated that within 30 minutes after hemorrhage there was a decrease in hydrogen-ion concentration, with an increase to above normal on the day following hemorrhage, and a return to normal a few days later. It has also been shown by other workers (Price-Jones, 1922; Smirk, 1928; Falik and Bielinski, 1931) that alterations in pH influence the size of cells, which increase in size with increased acidity and decrease with increased alkalinity. The decrease in mean corpuscular volume, noted in the present study on the second day following hemorrhage, would suggest an increased alkalinity of the blood, as reported by Bennett. On the other hand, this decrease in mean corpuscular volume could have resulted from recovery of the colloid osmotic pressure, since, as shown in the sections dealing with these fractions, plasma proteins and lipide phosphorus had in a large measure returned to normal 24 hours after bleeding.

Haden (1930), in reporting his work with human subjects, observed that changes in mean corpuscular volume were greater than changes in cell diameter. The data in the long-term study and on the three animals that were bled four to five times bear out Haden's observation, which is confirmed further by the fact that the correlation coefficient obtained shows no high degree of relationship between these two values during the post-bleeding period.

The extent to which body reserves of blood-building materials are depleted by hemorrhage influences the degree to which the cells are decreased in size, as is shown by the fact that in the study in which the animals were bled four or five times much greater decreases, both in cell diameter and in mean corpuscular volume, were observed than in the animals that were bled only twice, as in the long-term study. This is further borne out by the highly significant correlation coefficient secured between red cell count and cell diameter for the six animals used in the long-term study for which diameter data were available.

The more rapid the cell production, the smaller the cells produced.

Microcytosis was found in all of the experimental animals, indicating that in chronic blood loss dogs respond in this respect similarly to the way human subjects react (Price-Jones, 1922; Murphy and Fitzhugh, 1930; Haden, 1932; Wintrobe, 1929-30) rather than like rabbits, in which an increased cell diameter was observed (Ponder and Millar, 1928).

In the present investigation, the female animals displayed post-hemorrhagic decreases in cell size earlier than did the males, but except for this greater susceptibility on the part of the female animals, there was no essential difference in the character of the response.

Total Cell Volume

The factors limiting recovery following acute blood loss may be one or both of two kinds. There may be a reduced capacity for restoring or building cells, or there may be a reduced capacity for building hemoglobin. The lack of ability to build cells will be reflected also in the inability to restore the hemoglobin to the normal level. On the other hand, an animal's organism may be capable of restoring cells which in the absence of ability to restore hemoglobin will not be completely saturated with this pigment. In the present investigation an attempt was made to study not only the animals' capacity for building cells, but also their capacity for building hemoglobin.

In determining the volume of cells which an animal can produce following hemorrhage, due consideration should be given to the possible existence of a cell reserve which could be drawn into the blood stream. As pointed out in the section on cell counts, there was no evidence in the data acquired in the short-term study of a cell reserve in the animal body. This was confirmed by the results of observations made on total cell volume in that the mean total cell volume, measured both 30 minutes after the start of bleeding and 24 hours later, showed remarkable agreement with the predicted values based on the difference between the pre-bleeding normal mean total cell volume and the volume of cells removed in bleeding and sampling. The amount removed in daily sampling more than offset the amount the animals produced; hence there was a gradual decrease in total cell volume throughout the sampling period.

It is possible that inability to substantiate the existence of a reserve of preformed cells may have resulted from errors inherent in the dye method used for determining total cell volume. It has been reported by Smith, Arnold, and Whipple (1921) that the dye method produces values for total cell volume which are somewhat higher than the circulating cell volume in the animal body. This may tend to conceal the fact that reserve stores of cells actually exist. It would, however, necessarily mean that the reserve was essentially of the same magnitude as the error inherent in the dye method.

Although cell counts were restored to normal during the long-term study, owing to decrease in cell size, cell volume remained definitely below normal throughout the post-bleeding period. Cell count, therefore, apparently is not a dependable measure of the total volume of cells in a subject recovering from loss of blood.

When the post-bleeding period was divided into a "depletion" period representing the first two or three weeks following bleeding, and a "post-depletion" period representing the balance of the post-bleeding interval, evidence was found of the existence of reserves of cell-building materials in the fact that the animals were capable of much greater cell volume regeneration during the first two or three weeks following hemorrhage than they were during the remainder of the post-bleeding period. The fact that the males possessed appreciably greater reserves of cell-building materials than the females was shown by their more rapid cell volume regeneration during the depletion period. During the depletion period, the rate of regeneration for the males was 2½ times as rapid as during the post-depletion period, whereas for the females the rate of regeneration during the depletion period exceeded only by one third the regeneration noted for the post-depletion period. Apparently the reserve had been essentially exhausted, however, at the end of the depletion period because, although they continued to do slightly better than the females during the remainder of the period, the difference was small.

Wide individual differences in cell volume regeneration were apparent, there being animals within each group that apparently had high capacity, as well as some with low capacity, for cell volume regeneration. There is evidence that this was due to some condition inherent in the animals' physiological makeup, since there was a tendency for animals displaying a high rate of regeneration during the early stages to continue on a somewhat higher plane than the others during the post-depletion period.

Obviously these differences are not attributable to the size of the animals since all values were calculated on the basis of body weight. However, one contributing factor in cell volume regeneration during the post-depletion period was apparently the amount of the synthetic diet consumed, which observation was substantiated by the positive correlation coefficient obtained between food intake and cell volume regeneration. That it was not the only determinant was evident from the fact that the females actually consumed more food than the males on the basis of body weight although their cell volume regeneration, also on the basis of body weight, was distinctly lower.

The pre-bleeding mean normal cell volume was not a criterion of the animals' ability to replace lost blood cells. This conclusion was supported by the fact that those animals with a high normal cell volume per kilogram did not show a more rapid recovery than did the animals with a lower cell volume per kilogram.

Based on data secured in this investigation, plus unpublished data covering other experiments performed in this laboratory on an additional group of dogs, the factors that were observed to influence cell volume regeneration were the degree of physiological stress induced by bleeding, the reserve of cell-building materials in the animal body, and food intake.

Cell Volume Per Cent

The data secured in the present investigation covering cell volume per cent confirm the observations of Adolph, Gerbasi, and Lepore (1933), who found that dilution of the plasma with fluid drawn from extravascular spaces occurred rapidly following blood loss. They reported the process was essentially complete within 22 minutes after hemorrhage. In the present short-term study, the dilution process had not been completed within 30 minutes of the start of bleeding, although appreciable dilution had occurred within that time, followed by complete adjustment within the succeeding 24 hours.

Robertson and Bock (1919) suggested, from their experience with wounded soldiers, that the rate of recovery following blood loss seemed to be dependent upon the quantity of reserve fluids in the tissues, since if patients were given large quantities of fluid by alimentary tract the blood volume increased quickly.

In the present long-term study it was observed that cell volume per cent was influenced by the number of cells produced and the size of the individual cells, from which it followed that the animals which regenerated the greatest number of cells, and at the same time the largest cells, showed the greatest degree of recovery in cell volume per cent. The males showed superiority over the females in this respect.

Oxygen-combining Capacity

The current investigation demonstrated the fact that the total blood volume does not return to normal after loss of significant amounts of blood. Therefore, reduction in the total oxygen-combining capacity of the blood may be more serious than the oxygen capacity, in terms of volumes per cent, would indicate.

In the absence of blood volume data, either cell count or oxygen capacity will serve, during the early stages of recovery, as an equally good measure of an animal's progress toward normality. After a period of time, during which the body builds cells that are smaller than normal, cell counts may be high and, taken by themselves, create an erroneous conception of the extent of improvement. Since total cell volume may show no increase, on the contrary even a decrease, the oxygen-combining capacity of the blood, per unit of volume, is the better measure of progress.

The data secured in the short-term study showed the decreases in cell volume per cent and in oxygen-combining capacity in volumes per

cent to be of essentially the same magnitude, indicating that at least during the early stages of recovery the saturation of cells with hemoglobin was not altered following bleeding. The difficulties inherent in restoring hemoglobin to a normal level following loss of blood were evidenced by the fact that in the animals used in this study the level of oxygen capacity continued to decrease as a result of daily sampling.

In the long-term study also it was apparent that the weekly sampling, in which five per cent of the total blood volume was removed, largely offset any recovery that was made. The females actually lost ground in that their oxygen capacity was less at the end of the sixth post-bleeding week than it was at the end of the first week following hemorrhage. Although the males made somewhat better progress toward recovery than the females, their levels of oxygen capacity at the end of the sixth and at the end of the twelfth post-bleeding week were very little above the value observed at the end of the first post-bleeding week.

It was also evident from the results of this research, plus unpublished data covering other experiments performed in this laboratory on an additional group of dogs, that the animals which had the highest pre-bleeding normal oxygen capacity were the furthest from their own normal levels at the end of the sixth post-bleeding week. The normal oxygen capacity of the blood is, therefore, plainly no indication of an animal's reserve of hemoglobin-building materials.

Recovery of the oxygen capacity of the blood is possibly related to the severity of the anemia produced. There is doubtless a critical level above which the oxygen capacity of the blood must be maintained even at the expense of other body tissues. Animals whose normal oxygen capacity is low will fall much more readily below this critical level; hence with such animals there is greater physiological need for recovery than with animals whose normal oxygen capacity is high. In other words, the loss of half the total blood volume in dogs with a high normal oxygen capacity will not serve as a stimulus to erythropoiesis in the same degree as in animals whose normal oxygen capacity is low.

Most workers in this field use the acid-hematin method rather than the more time-consuming oxygen-capacity method for observing the changing blood picture. Therefore, for the purpose of comparing the results secured in the present investigation with those reported by other workers, recovery in the oxygen-combining capacity of the blood was calculated in terms of grams of hemoglobin regenerated.

During the depletion period it was apparent that the males did possess some store of hemoglobin-building materials since they were capable of producing more than twice the amount of hemoglobin per kilogram per week during this period than they were during the remainder of the post-bleeding interval. The females, on the other hand, showed no such reserve, their rate of regeneration during the depletion period being actually slightly less than during the remainder of the post-bleeding interval.

The results cited above are contrary to the findings of Smith and Otis (1937) and Hubbell and Rose (1937), who in reporting studies on nutritional anemia in rats stated that female rats possessed greater reserves of hemoglobin-building materials than did the males.

It must be recognized that the earlier histories of the dogs used in this investigation were unknown; therefore, it is quite possible that the females had experienced one or more pregnancies which might have depleted or exhausted the reserves of hemoglobin-building materials.

The data obtained in the current research are in accord with results reported on observations made on male and female blood donors in that female donors are much less satisfactory than males. They develop anemia more quickly and also experience greater difficulty in recovery (Jones, Widing, and Nelson, 1931; Martin and Myers, 1934-35).

Some hemoglobin regeneration was observed on the synthetic diet, but no evidence was secured to indicate that the rate of regeneration was correlated with the level of food intake. Although the females actually consumed larger quantities of food per kilogram of body weight, their rate of hemoglobin regeneration was lower than that of the males.

Whipple and his co-workers conducted a long and comprehensive series of experiments on dogs to ascertain the effect of various foods on hemoglobin regeneration. Using a salmon-and-bread mixture as their basal diet, they observed a hemoglobin regeneration of 1.0 to 2.0 g. per two-week period. When this basal diet was supplemented with inorganic iron salts, Whipple and Robscheit-Robbins (1930) observed a maximum effect from the addition of 40.0 mg. of iron daily. When they used ferric citrate as the iron salt, they noted an increase in hemoglobin production amounting to 25.0 g. per week.

In the present investigation, the mean daily iron intake, in the form of ferric citrate, was 21.6 mg. for the females and 24.6 mg. for the males. The mean hemoglobin production for the females per week was 4.7 g., and for the males, 7.5 grams. Since the iron intake of the females was only 54 per cent, and of the males only 62 per cent of the amount of iron which Whipple and Robscheit-Robbins stated was the optimal level for hemoglobin production in dogs, it would be anticipated that the hemoglobin production for the animals used in the present studies would be proportionately less. On this basis the hemoglobin production expected of the females would have been 13.5 g., and of the males, 15.4 g. per week. Actually, the hemoglobin production of the females was only one third of this predicted amount, and of the males, only one half. These results would suggest that the limiting factor in the hemoglobin production in these animals was not the iron intake, but rather some factor other than iron needed for hemoglobin production which was supplied in more adequate amounts by the Whipple and Robscheit-Robbins' bread-and-salmon diet than by the synthetic diet used in the present investigation.

McKay (1928) observed slow hemoglobin regeneration on a synthetic diet similar to that used in the present research, while Mayerson and Laurens (1930-31) found Cowgill's synthetic diet inadequate for maintaining a constant hemoglobin level in their anemic animals. None of these latter investigators supplemented their salt mixtures with copper as was done in the current investigation, which may have been an additional limiting factor in their experiments.

Leucocytes

In the present research there appeared to be a mild leucocytosis following hemorrhage similar to that reported by Keith (1919) and Jolly (1923). The latter suggested that the increase in leucocytes might be a temporary phenomenon, appearing shortly after hemorrhage, but not necessarily of uniform occurrence. The increase observed in this study, however, was not statistically significant when subjected to Student's "t" test.

In the short-term study, possibly the determination made within 30 minutes of the start of bleeding did not allow sufficient time for increases to occur since the change which was actually observed at that time was a decrease in leucocytes. This was interpreted as resulting from the rapid dilution of the plasma with interstitial fluid. The slight increases in the mean values observed on the days following hemorrhage resulted in a large measure from increases noted for the one animal, number 47, on which four experiments were performed.

In the long-term study the females displayed more consistent evidence of a mild leucocytosis during the post-hemorrhagic period than did the males, but even among the females such evidence was not always present. Again, the observed increases were not found to be statistically significant.

Mayerson and Laurens (1930-31) reported that in only two of their experimental animals did they observe marked or consistent changes in either total white or differential counts. In them they noted a decrease in lymphocytes and a corresponding increase in neutrophiles.

In the third study, in which the animals were bled repeatedly, there was a very small increase in total neutrophiles, owing largely to a small but significant increase in the young band forms ($P = <0.023$). A corresponding decrease was observed in the mean values for total lymphocytes owing to decreases in both small and large forms.

SUMMARY

Erythrocyte Counts

In the short-term study, a 20 per cent decrease in erythrocyte counts was observed 24 hours after removing 26 per cent of the total blood volume. A continued decrease resulted thereafter from daily sampling.

Data on cell counts produced no evidence of a reserve of preformed cells which could be drawn into the circulation in an emergency. In the long-term study, after removing approximately half of the total blood volume, the males' red cell counts had returned to normal in five weeks and the females', in seven weeks, after which counts for both males and females were distinctly above normal despite weekly sampling.

Cell Size

In the short-term study, mean corpuscular volume determinations, 30 minutes after the start of bleeding, produced a mean which was slightly above the pre-bleeding value, followed by decreases during the post-bleeding period to a level slightly below the pre-bleeding mean, five to seven days after hemorrhage. In the long-term study, cell diameter decreased throughout the first six weeks of the post-bleeding period, the females showing a more rapid decrease than the males. On a percentage basis, the decrease was 10 per cent for the females and 9 per cent for the males. Further small decreases were noted during the second six-week portion of the post-bleeding period. During the first six weeks of the post-bleeding period mean corpuscular volume decreased 17 per cent for the females, 15 per cent for the males. Further decreases during the balance of the post-bleeding period were observed for the females but not for the males. In a third study, on dogs bled four to five times and then observed for approximately four months, cell diameter decreased 24 per cent and mean corpuscular volume, 29 per cent.

Total Cell Volume

Based on total cell volume, also, no evidence was secured in the short-term study to substantiate the existence of a reserve of preformed cells. Cell volume per kilogram decreased throughout the post-bleeding period as a result of daily sampling and reduction in cell size. In the long-term study, the females' cell volume per kilogram showed very little recovery during the first six weeks of the post-bleeding period following the initial decrease after the hemorrhages. For the males, recovery was somewhat more rapid. Neither males nor females made much progress during the remainder of the post-bleeding period.

The animals were fed a synthetic ration in amounts needed to maintain relatively constant body weight. Despite the fact that the mean food intake for the females was higher than for the males, the males showed more satisfactory progress during the recovery period than did the females.

The percentage of total blood volume removed from the males was slightly higher than that removed from the females, showing that the males were handled as drastically as the females in the matter of bleeding and sampling.

The mean volume of cells regenerated during the first six weeks of the post-bleeding period was appreciably greater for the males than for the females, wide variations from animal to animal being evident in both groups. A significant positive correlation coefficient, +.6152, between the percentage of cells removed and cell volume regenerated, indicates that physiological stress induced by bleeding influences cell volume regeneration.

Although the males appeared to possess greater stores of cell-building materials than did the females as shown by the fact that, based on body weight, they regenerated during the first two to three weeks following hemorrhage more than twice the volume of cells the females produced, the difference did not prove to be statistically significant. The exhaustion of these reserves was evidenced by the reduced capacity of both males and females to regenerate cell volume during the remainder of the post-bleeding period, the males again showing slightly higher values than the females. Differences in the capacities of individual animals were apparent even after the exhaustion of cell reserves.

A significant positive correlation coefficient, +.7873, between the volume of cells regenerated per kilogram of body weight and food intake per kilogram proved that during the later stages of recuperation cell volume regeneration was in part controlled by food intake.

An insignificant correlation coefficient showed that no relationship existed between normal cell volume and the volume of cells regenerated in cubic centimeters per kilogram.

Cell Volume Per Cent

In the short-term study, the rapidity with which interstitial fluid was drawn into the blood stream following hemorrhage was demonstrated by an appreciable drop in cell volume per cent within 30 minutes after the start of hemorrhage. Complete adjustment followed within 24 hours. Further small decreases occurred throughout the remainder of the experiment owing to daily sampling and reduction in cell size. In the long-term study, the slow increase in cell volume per cent reflected the retarded recovery in total cell volume, the males making slightly better progress during the first six weeks of the post-bleeding period than the females. Neither males nor females showed much improvement during the second six-week portion of the post-bleeding period.

Interrelationships between Red Cell Count, Size, and Volume of Cells

In the long-term study, a significant correlation coefficient was obtained between cell count and cell diameter, —.7745, indicating a close relationship between cell diameter and the number of cells regenerated in hemorrhagic anemia, and thus verifying the earlier findings of Leichsenring and Hönig (1931).

The significant correlation coefficient, +.4411, between cell diameter and mean corpuscular volume showed no high degree of relationship between these two factors.

The correlation coefficient between cell count and cell volume in cubic centimeters per kilogram, +.3524, while significant, indicated that cell count is not an entirely satisfactory measure of total cell volume.

Oxygen-combining Capacity

In the short-term study, the decrease observed 24 hours after hemorrhage was in close agreement with the drop in cell volume per cent. Although values noted on succeeding days were not in quite such close agreement, the data suggest that the concentration of hemoglobin in the cell is unaltered in the early stages of recuperation. In the long-term study, whereas the males at the end of the sixth post-bleeding week had made some progress toward recovery, the females actually showed a loss. Neither males nor females made any further progress during the second six-week interval. A significant correlation coefficient, +.5458, between the normal oxygen capacity of the blood and the level at the end of the sixth post-bleeding week demonstrated the fact that the animals with the highest normal oxygen capacity were the furthest from their own normal levels at the end of this interval.

Recovery in oxygen capacity was calculated in terms of grams of hemoglobin regenerated. During the first two to three weeks of the post-bleeding period the males regenerated more than twice as much hemoglobin per kilogram per week as during the remainder of the post-bleeding period. The females, on the other hand, did not demonstrate a reserve of hemoglobin-building materials. The males showed superiority over the females during the entire post-bleeding period. Hemoglobin regeneration was not related to the level of food intake as shown by the insignificant correlation coefficient obtained between these two factors.

Leucocytes

In the short-term study, the apparent leucocytosis noted in the post-bleeding period was shown to be statistically insignificant. In the long-term study, both females and males showed some evidence of leucocytosis, maximum counts being observed at the end of the first and second post-bleeding weeks, respectively, with decreases toward normal levels throughout the remainder of the post-bleeding period. Again the observed increase was not significant.

In the third study, the animals showed no increase in the total leucocytes after repeated hemorrhage. The differential leucocyte count showed a small but significant increase in the percentage of the young band-form neutrophiles ($P = <0.023$), accompanied by a decrease in the total lymphocytes involving both small and large forms.

V. BLOOD PLASMA

PLASMA VOLUME

IN THE DISCUSSION of cell volume per cent, from data secured in the short-term study, it was pointed out that there was a rapid withdrawal of interstitial fluid from the tissues to restore plasma volume to normal. Within 30 minutes of the start of hemorrhage, in which 20 per cent of the total blood volume had been removed, the mean plasma volume was observed to be 773.5 cc., compared with a pre-bleeding mean of 889.0 cc., a decrease of only 13 per cent. When these volumes were calculated on the basis of the mean body weight of the animals, the level 30 minutes after the start of bleeding was 39.7 cc. per kilogram, as against a mean pre-bleeding value of 45.0 cc. per kilogram (table 35). Equilibrium had been established 24 hours later at a level slightly above the pre-bleeding level (46.0 cc. per kilogram), and thereafter increases in plasma volume per kilogram tended to balance the losses in cell volume owing to daily sampling. However, at no time did the increase in plasma volume compensate completely for the loss in cells, with the result that the total blood volume remained distinctly below normal throughout the post-bleeding period (table 50).

Table 35. Values for Plasma Volume, in Cubic Centimeters per Kilogram (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	46.6	41.9	46.7	47.4	46.4	46.0
	48.3	37.3	45.4	45.4	46.6	48.3
45	39.8	.....	44.0	44.5	45.8	44.6
	41.5	33.0	43.3	42.2	42.9	44.5
46	47.1	42.2	52.4	50.1	54.2	49.2
	48.6	39.4	45.3	48.9	50.8	51.3
47	39.1	40.5	42.4	37.6	37.5	44.6
	45.9	43.3	45.4	48.5	49.7	49.8
	47.1	39.2	43.4	46.0	43.4	44.9
	46.0	40.6	51.2	47.0	50.5	50.2
Mean	45.0	39.7	46.0	45.8	46.8	47.3

For the female dogs used in the long-term study, the mean normal plasma volume was 35.3 cc. per kilogram (table 36). One week after the removal of approximately one half the established total blood volume, plasma volume had increased to 38.3 cc., with thereafter a gradual de-

crease until at the end of six weeks the mean plasma volume was found to be 36.6 cc. per kilogram. For the three females observed for 12 weeks, the mean at the end of the twelfth week was 36.7 cc., contrasted with a mean of 37.8 cc. at the end of the sixth week.

Table 36. Values for Plasma Volume, in Cubic Centimeters per Kilogram (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-5 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	41.4	40.6	44.5	41.4	44.2	39.7	42.0	45.1	44.4
21	37.7	47.3	38.6	42.3	32.2	35.4	37.0	37.9	.....
22	33.8	38.7	33.1	36.0	36.8	34.3	34.1	32.0	.....
19	28.9	41.7	34.0	34.2	.....	37.8	33.0	32.2	30.6
32	34.8	37.5	41.4	38.1	38.1	37.6	37.2	36.0	35.0
Mean	35.3	41.2	38.3	38.4	37.8	37.0	36.7	36.6	36.7
Males									
17	37.2	34.8	41.3	41.7	40.0	37.8	35.7	40.9	46.0
18	31.5	38.1	33.3	30.6	37.6	38.2	34.8	35.1	36.0
30	45.9	49.6	49.0	48.7	47.2	43.2	46.7	46.4	40.2
31	41.0	41.6	44.7	43.0	41.7	40.2	40.4	40.4	38.1
Mean	38.9	41.0	42.1	41.0	41.6	39.8	39.4	40.7	40.0

The mean normal plasma volume for the males in this group was 38.9 cc. per kilogram, a somewhat higher value than that observed for the females. The plasma volume had reached a mean level of 42.1 cc. by the end of the first post-bleeding week, 40.7 cc. by the end of the sixth week, with a further slight decrease to 40.0 cc. by the end of the twelfth week. Evidently the animal body is capable of adjusting to an appreciably smaller total blood volume following hemorrhage.

BLOOD PLASMA CONSTITUENTS

Protein Nitrogen.—In the short-term study, the plasma protein nitrogen, measured in grams per 100 cubic centimeters, did not return to normal during the post-bleeding period (table 37). The mean pre-bleeding value was 1.07 g. per 100 cc. of plasma. Within 30 minutes of the start of hemorrhage the mean was 1.00 g., and throughout the remainder of the post-bleeding period the values observed were slightly below this level.

Multiplying the grams of protein nitrogen per cubic centimeter by the total plasma volume in cubic centimeters, to secure the total plasma protein nitrogen in the total circulation, a value was obtained which showed a return to normal 24 hours after hemorrhage (table 38). The pre-bleeding mean for the total protein nitrogen was 9.02 g., compared with the first post-bleeding value, secured 24 hours after hemorrhage,

Table 37. Values for Protein Nitrogen in Plasma, in Grams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	1.040	0.986	1.049	1.001	0.978	1.003
	1.085	1.060	1.025	1.026	0.979	0.981
45	1.089	.....	1.050	0.922	0.865	0.959
	1.042	1.034	.....	0.945	0.959	0.959
46	1.140	1.104	1.064	1.072	1.038	1.007
	1.047	1.005	1.005	0.982	1.032	0.971
47	1.062	0.819	0.941	0.944	0.924	0.874
	1.066	1.019	0.953	0.971	1.042	1.024
	1.072	0.974	0.922	1.026	1.015	1.018
	1.046	.....	0.978	1.015	1.001	1.000
Mean	1.069	1.000	0.996	0.990	0.983	0.980

Table 38. Comparison of the Mean Total Amounts of Each of the Plasma Constituents in the Circulation Before and After Hemorrhage* (Short-term study)

		Nitrogen		Calcium			Phosphorus		
		Protein in grams	Non-protein in grams	Diffusible in mg.	Non-diffusible in mg.	Total in mg.	Lipide in mg.	Inorganic in mg.	Total in mg.
Pre-bleeding values	1	8.60	0.228	62.8	44.9	107.7	135.4	41.4	184.2
	2	8.86	0.211	65.4	37.6	96.0	137.0	44.8	194.2
	3	9.31	0.229	66.0	35.6	101.6	150.8	47.4	212.2
	4	9.31	0.225	70.4	42.1	112.5	155.3	46.2	209.7
Mean		9.02	0.223	66.2	40.4	104.4	144.6	45.0	200.1
Within 30 minutes of start of hemorrhage		7.96	0.197	57.6	36.1	93.7	122.0	38.7	182.7
Post-bleeding values	1	9.06	0.221	67.9	47.4	115.3	155.4	45.6	196.4
	2	9.03	0.209	67.8	45.4	113.2	155.6	46.9	211.0
	3	9.20	0.226	67.0	40.4	107.4	152.7	48.4	209.2
	4	9.18	0.224	71.5	40.9	112.4	152.0	46.3	210.1
	5	9.52	0.229	71.0	46.5	117.5	152.4	50.2	217.2
Mean		9.20	0.222	69.0	44.1	113.2	153.6	47.5	208.8

* Each value represents the mean result of the 10 experiments, and was obtained by multiplying the amount per cubic centimeter by the plasma volume in cubic centimeters. The values obtained on samples secured within 30 minutes of the institution of bleeding were not included in the post-bleeding mean.

of 9.06 g., and a mean of 9.20 g. for the post-bleeding period. Considered solely on the basis of the amount of protein nitrogen per unit of volume, this recovery is not apparent because of the increase in plasma volume.

Nonprotein Nitrogen.—As with the protein nitrogen, the nonprotein nitrogen level was slightly below the pre-bleeding normal during the post-bleeding period (table 39). The mean pre-bleeding value obtained was 27.0 mg. per 100 cc. plasma. A similar mean value was observed again within 30 minutes of the start of hemorrhage, 26.7 mg.,

Table 39. Values for Nonprotein Nitrogen in Plasma, in Milligrams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	20.7	21.6	20.4	18.9	18.7	18.6
	19.3	20.3	18.1	18.0	17.2	18.3
45	31.8	32.1	31.4	26.0	28.6	30.9
	22.5	20.5	24.2	22.6	22.3	22.8
46	32.8	28.4	23.7	24.0	26.7	25.0
	25.5	26.6	22.0	25.1	27.7	24.1
47	33.9	31.6	33.3	29.0	26.6	24.4
	34.6	32.9	29.1	27.5	27.9	29.5
	26.8	28.6	27.3	24.4	27.4	27.9
	22.1	24.5	24.5	20.8	22.8	20.7
Mean	27.0	26.7	25.4	23.6	24.6	24.2

with a decrease to 25.4 mg. 24 hours later, to a still lower value, 23.6 mg., followed by slight variations throughout the balance of the post-bleeding period. When, as in the case of the protein nitrogen, the total amount of nonprotein nitrogen in the circulation was calculated, a surprising agreement was noted between pre-bleeding and post-bleeding means, the value for the former being 0.223 g., for the latter, 0.222 g., in the total plasma (table 38).

In the long-term study, determinations on total nitrogen and nonprotein nitrogen were made on whole blood. The resulting values will, therefore, be discussed in the section to follow.

Phosphorus and Calcium Fractions.—Although lipide phosphorus per unit volume of plasma decreased within 30 minutes of the start of bleeding, and then returned almost to normal within 24 hours, this level was not maintained throughout the post-bleeding period. The mean pre-

Table 40. Values for Lipide Phosphorus in Plasma, in Milligrams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	18.89	18.60	18.45	18.83	16.65	17.17
	17.75	15.53	17.64	17.94	15.01	16.68
45	19.95	18.84	18.68	20.35	17.50	18.46
	18.15	16.07	18.56	14.97	16.78	15.26
46	13.59	13.16	12.97	12.62	14.28	13.17
	9.70	10.59	10.58	12.62	12.56	10.16
47	17.33	14.37	18.44	16.87	20.22	18.50
	18.09	16.43	18.72	18.52	.....	19.58
	21.16	18.81	20.36	19.40	19.66	19.48
	19.97	16.72	19.21	19.12	18.55	18.15
Mean	17.46	15.91	17.36	17.12	16.80	16.66

bleeding level was 17.5 mg. per 100 cc., which decreased to 15.9 mg., and recovered to 17.4 mg. 24 hours after hemorrhage (table 40). On the second and third days, the values noted were 17.1 and 16.8 mg., respectively.

The level for inorganic phosphorus showed, within 30 minutes of the start of bleeding, the effect of dilution similar to that observed in the other factors studied (table 41). During the balance of the week, the

Table 41. Values for Inorganic Phosphorus in Plasma, in Milligrams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	6.49	6.01	5.79	6.53	5.67	5.96
	5.76	5.36	6.13	5.93	5.43	5.62
45	6.93	5.13	5.55	5.22	5.49	5.17
	5.84	5.05	5.27	4.65	5.46	4.95
46	5.93	4.71	4.46	4.99	5.08	4.96
	4.51	5.10	4.49	4.62	4.86	4.21
47	4.04	3.72	4.25	4.15	3.64	4.34
	4.72	4.49	4.10	4.80	.....	5.54
	5.06	4.20	4.98	4.96	4.88	5.20
	5.79	5.00	4.77	4.82	4.90	4.12
Mean	5.51	4.88	4.98	5.07	5.04	5.01

values were slightly below the value obtained for the pre-bleeding sample. The mean pre-bleeding value for the 10 experiments was 5.51 mg. per 100 cc., which decreased to 4.88 mg. within 30 minutes of the start of bleeding, and remained approximately unchanged for the balance of the post-bleeding period.

Total phosphorus data for these animals are given in table 42. It will be noted that the means are in all cases slightly higher than the

Table 42. Values for Total Phosphorus in Plasma, in Milligrams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	26.85	24.74	23.89	25.00	23.78	23.84
	23.54	22.49	24.91	23.77	22.49	22.36
45	28.08	24.31	24.86	27.38	24.66	24.62
	23.37	21.05	23.61	18.99	20.59	21.24
46	20.76	19.00	20.34	20.17	20.35	19.86
	17.00	16.69	18.14	17.45	19.38	16.59
47	20.46	17.67	21.35	20.62	26.40	22.02
	24.42	21.49	22.76	25.14	23.70	26.12
	26.23	23.50	26.12	25.66	24.80	27.34
	26.36	22.89	24.60	23.40	23.45	23.14
Mean	23.71	21.38	23.06	22.76	22.96	22.71

sum of the means for the lipide and inorganic phosphorus fractions, indicating the presence in the blood, in relatively constant amounts, of other phosphorus-containing compounds.

The means for diffusible and nondiffusible calcium, observed within 30 minutes of the start of bleeding, also show the effects of dilution. For diffusible calcium, the mean pre-bleeding value was 7.71 mg. (table 43), which decreased within this 30-minute interval to 7.33 milligrams. During the rest of the week values noted were slightly below the pre-bleeding level, namely, 7.44, 7.40, 7.10, and 7.60 mg., respectively.

The mean pre-bleeding value for nondiffusible calcium was 5.00 mg. per 100 cc. of plasma (table 44), which decreased within 30 minutes after the start of bleeding to 4.88 milligrams; 24 hours thereafter a mean of 5.42 mg. was obtained, followed on the second day by a mean value of 4.99 milligrams. Although the extent to which these values were influenced by the loss of one sample on each of the first three post-bleeding days cannot be determined, there appears to be a clear tendency for the level of the nondiffusible calcium to decrease after the first post-bleeding day to a value below the pre-bleeding mean.

Table 43. Values for Diffusible Calcium in Plasma, in Milligrams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	8.82	8.16	6.82	7.66	8.26	8.57
	6.29	6.03	6.27	6.11	5.98	6.29
45	8.70	7.57	8.54	7.66	7.68	7.53
	7.69	7.50	9.29	8.14	8.31	8.37
46	7.87	7.82	7.80	7.93	8.19	8.45
	8.45	8.58	6.66	6.82	6.25	6.69
47	5.92	6.31	6.53	6.78	5.87	6.55
	9.47	7.72	7.64	8.04	7.30	8.29
	7.80	7.83	7.98	7.96	7.39	7.76
	6.08	5.78	6.87	6.95	5.74	7.49
Mean	7.71	7.33	7.44	7.40	7.10	7.60

Again, as in the case of the protein nitrogen and nonprotein nitrogen, if the total amount of these various fractions in the circulating plasma were calculated by multiplying the amount of each, per unit of volume, by the total volume of the plasma, a complete recovery of the total amount in the circulation would be observed (table 38). In fact, for all fractions, slightly higher means were apparent in the post-bleeding period when compared with the pre-bleeding values. The pre-bleeding and post-bleeding totals for the various factors in the circulating plasma

Table 44. Values for Nondiffusible Calcium in Plasma, in Milligrams per 100 Cubic Centimeters (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	5.44	5.93	6.83	.....	3.86	4.73
	4.80	5.42	4.90	6.37	5.47	3.80
45	6.71	6.49	4.05	6.59	4.95	4.77
	4.97	3.56	.....	3.19	3.21	4.58
46	4.37	3.49	4.68	4.58	4.28	4.21
	3.54	4.47	4.09	3.92	3.81	4.48
47	5.39	5.00	6.66	4.95	4.02	4.26
	4.76	5.81	6.74	5.16	.....	4.53
	4.49	4.01	5.76	4.33	5.20	4.44
	5.55	4.57	5.06	5.84	4.11	4.62
Mean	5.00	4.88	5.42	4.99	4.32	4.44

follow: lipide phosphorus, 144.6 and 153.6 milligrams; inorganic phosphorus, 45.0 and 47.5 milligrams; total phosphorus, 200.1 and 208.8 milligrams; diffusible calcium, 66.2 and 69.0 milligrams; nondiffusible calcium, 40.4 and 44.1 milligrams; and total calcium, 104.4 and 113.2 milligrams, respectively.

Significant positive correlations were obtained between the levels of plasma protein and inorganic phosphorus before hemorrhage, +.3248, and after hemorrhage, +.3487.

After hemorrhage, significant positive correlations were observed between lipide phosphorus and nonprotein nitrogen, +.2575; between lipide phosphorus and inorganic phosphorus, +.4371; between lipide phosphorus and diffusible calcium, +.3077; and between inorganic phosphorus and diffusible calcium, +.3897, although no such relationships were apparent in the pre-bleeding levels.

A significant negative correlation was obtained between pre-bleeding values for diffusible and nondiffusible calcium, —.3787, but not between the post-bleeding values.

Plasma Protein Fractions: Albumin.—In the fourth study, working with three male dogs, the effect of repeated bleeding on the level of the plasma proteins was investigated. It was observed that the level of albumin in the plasma was restored rapidly. The mean normal level was 3.98 g. per 100 cc. of plasma (table 45), but 48 hours after the loss of one fourth to one half of the total blood volume (in one or two bleedings) the mean level was found to be 4.05 grams. Although each animal was bled two or three times more, removing at each bleeding one fourth of the total blood volume, at no time was the mean albumin level found to be below normal. As a matter of fact, a mean distinctly higher than normal, 4.70 g., was obtained following the third hemorrhage.

Globulin.—The globulin level, on the other hand, was apparently restored with difficulty (table 46). The mean normal value was found to be 2.46 g. per 100 cc. of plasma. Forty-eight hours after the first hemorrhage the mean value was 1.89 g., and after succeeding hemorrhages, mean values of 2.17 and 2.00 g. were noted. Two of the animals were subjected to a fourth bleeding. In these dogs, whose normal levels produced a mean of 2.14 g., the globulin level increased after each hemorrhage as shown by the means—1.84, 2.04, 2.24, and 2.50 grams. The results cited indicate that the animal organism evidently can adapt itself to losses of globulin by increasingly rapid regeneration and finally attain a value actually higher than normal.

Albumin-Globulin Ratio.—Alterations in the albumin-globulin ratio, corresponding with these changing levels, ranged from a mean pre-bleeding normal of 1.69 to a mean of 2.53 following the third hemorrhage (table 47).

Table 45. Albumin in Plasma, in Grams per 100 Cubic Centimeters

Dog No.	Pre-bleeding normal	After first hemorrhage		After second hemorrhage		After third hemorrhage		After fourth hemorrhage	
		No. days	Amount	No. days	Amount	No. days	Amount	No. days	Amount
Males									
55	3.78	2	3.97	2	2.98	2	4.66	3	4.08
	3.59	.....	.....	7	3.70	10	4.42	10	4.32
	.....	.....	.....	14	4.19	17	4.12	.....	.....
	.....	.....	.....	.....	.....	24	4.47	.....	.....
Mean	3.69	.....	3.97	.....	3.62	.....	4.42	.....	4.20
54	4.01	2	3.89	2	3.60	3	4.62	2	4.05
	.....	.....	.....	9	4.14	10	4.41	8	4.42
	.....	.....	.....	16	4.23	.....	.....	.....	.....
Mean	4.01	.....	3.89	.....	3.99	.....	4.52	.....	4.24
56	3.98	2	4.28	2	4.09	2	5.03	.....	.....
	4.52	.....	.....	9	4.56	7	5.26	.....	.....
	.....	.....	.....	14	4.59	.....	.....	.....	.....
Mean	4.25	.....	4.28	.....	4.41	.....	5.15	.....	.....
Mean for 3 dogs	3.98	.....	4.05	.....	4.01	.....	4.70	.....	4.22

Table 46. Globulin in Plasma, in Grams per 100 Cubic Centimeters

Dog No.	Pre-bleeding normal	After first hemorrhage		After second hemorrhage		After third hemorrhage		After fourth hemorrhage	
		No. days	Amount	No. days	Amount	No. days	Amount	No. days	Amount
Males									
55	2.66	2	1.48	2	2.03	2	2.22	3	2.11
	2.22	.....	.....	7	1.60	10	1.95	10	2.51
	.....	.....	.....	14	2.04	17	2.41	.....	.....
	.....	.....	.....	.....	.....	24	2.42	.....	.....
Mean	2.44	.....	1.48	.....	1.89	.....	2.25	.....	2.31
54	1.84	2	2.21	2	2.50	3	2.02	2	2.72
	.....	.....	.....	9	2.08	10	2.44	8	2.69
	.....	.....	.....	16	1.97	.....	.....	.....	.....
Mean	1.84	.....	2.21	.....	2.18	.....	2.23	.....	2.70
56	2.83	2	1.98	2	2.29	2	1.17	.....	.....
	3.36	.....	.....	9	2.80	7	1.85	.....	.....
	.....	.....	.....	14	2.22	.....	.....	.....	.....
Mean	3.09	.....	1.98	.....	2.44	.....	1.51	.....	.....
Mean for 3 dogs	2.46	.....	1.89	.....	2.17	.....	2.00	.....	2.51

Table 47. Albumin-Globulin Ratio in Plasma

Dog No.	Pre-bleeding normal	After first hemorrhage		After second hemorrhage		After third hemorrhage		After fourth hemorrhage	
		No. days	Amount	No. days	Amount	No. days	Amount	No. days	Amount
Males									
55	1.42	2	2.68	2	1.47	2	2.10	3	1.93
	1.62	.....	.....	7	2.31	10	2.27	10	1.72
	.....	.....	.....	14	2.05	17	1.71	.....	.....
	.....	.....	.....	.....	.....	24	1.85	.....	.....
Mean	1.52	.....	2.68	.....	1.94	.....	1.98	.....	1.83
54	2.18	2	1.76	2	1.44	3	2.29	2	1.49
	.....	.....	.....	9	1.99	10	1.81	8	1.64
	.....	.....	.....	16	2.15	.....	.....	.....	.....
Mean	2.18	.....	1.76	.....	1.86	.....	2.05	.....	1.57
56	1.41	2	2.16	2	1.79	2	4.30	.....	.....
	1.34	.....	.....	9	1.63	7	2.84	.....	.....
	.....	.....	.....	14	2.07	.....	.....	.....	.....
Mean	1.37	.....	2.16	.....	1.83	.....	3.57	.....	.....
Mean for 3 dogs	1.69	.....	2.20	.....	1.88	.....	2.53	.....	1.70

Table 48. Osmotic Pressure of Plasma Proteins, in Millimeters of Mercury (Calculated on Basis of Govaerts' Factors)

Dog No.	Pre-bleeding normal	After first hemorrhage		After second hemorrhage		After third hemorrhage		After fourth hemorrhage	
		No. days	Amount	No. days	Amount	No. days	Amount	No. days	Amount
Males									
55	24.4	2	23.8	2	19.2	2	28.6	3	25.3
	22.8	.....	.....	7	22.5	10	27.0	10	27.2
	.....	.....	.....	14	25.8	17	25.9	.....	.....
	.....	.....	.....	.....	.....	24	27.9	.....	.....
Mean	23.6	.....	23.8	.....	22.5	.....	27.4	.....	26.2
54	24.7	2	24.4	2	23.2	3	28.1	2	26.0
	.....	.....	.....	9	25.6	10	27.6	8	28.0
	.....	.....	.....	16	26.5	.....	.....	.....	.....
Mean	24.7	.....	24.4	.....	25.1	.....	27.8	.....	27.0
56	25.8	2	26.2	2	25.6	2	29.2	.....	.....
	29.5	.....	.....	9	28.9	7	31.4	.....	.....
	.....	.....	.....	14	28.2	.....	.....	.....	.....
Mean	27.6	.....	26.2	.....	27.6	.....	30.3	.....	.....
Mean for 3 dogs	25.3	.....	24.8	.....	25.1	.....	28.5	.....	26.6

Since the plasma proteins contribute to the maintenance of the osmotic pressure of the blood, it was interesting to note the extent to which changes in the protein levels influenced the change in osmotic pressure. Govaerts (1925, 1926) had suggested factors to be used in determining the osmotic pressure contributed by the plasma proteins. Based on these factors, the calculated mean normal osmotic pressure contributed by the albumin and globulin was 25.3 millimeters of mercury (table 48).

Following the first hemorrhage, the mean was slightly below normal, 24.8 millimeters, but after the second hemorrhage it increased slightly

Table 49. Fibrinogen in Plasma, in Grams per 100 Cubic Centimeters

Dog No.	Pre-bleeding normal	After first hemorrhage		After second hemorrhage		After third hemorrhage		After fourth hemorrhage	
		No. days	Amount	No. days	Amount	No. days	Amount	No. days	Amount
Males									
55	0.11	2	0.17	2	0.24	2	0.28	3	0.28
	0.23	.....	.....	7	0.26	10	0.35	10	0.23
	.....	.....	.....	14	0.25	17	0.38	.....	.....
	.....	.....	.....	.....	.....	24	0.28	.....	.....
Mean	0.17	.....	0.17	.....	0.25	.....	0.32	.....	0.26
54	0.33	2	0.34	2	0.27	3	0.30	2	0.33
	.....	.....	.....	9	0.35	10	0.33	8	0.30
	.....	.....	.....	16	0.33	.....	.....	.....	.....
Mean	0.33	.....	0.34	.....	0.32	.....	0.32	.....	0.32
56	0.25	2	0.20	2	0.24	2	0.23	.....	.....
	0.31	.....	.....	9	0.23	7	0.23	.....	.....
	.....	.....	.....	14	0.20	.....	.....	.....	.....
Mean	0.28	.....	0.20	.....	0.22	.....	0.23	.....	.....
Mean for 3 dogs	0.26	.....	0.24	.....	0.26	.....	0.29	.....	0.29

to a level of 25.1 millimeters, the latter value representing a mean of three determinations on each of the three animals. Calculation of the mean for the first determination on each of the three dogs gave a value of 22.7 millimeters. Similar calculations for the second and third determinations produced means of 25.7 and 26.8 millimeters, respectively, showing a gradual rise in osmotic pressure during the period following the second hemorrhage.

Following the third hemorrhage, the osmotic pressure contributed by the plasma proteins was decidedly above normal, 28.5 millimeters of mercury. This value also represented a mean of two to four determinations on each of the three animals. The mean of the first determinations was 28.6 millimeters of mercury; of the second, 28.7. It is evident, therefore, that there was no apparent increase in osmotic pressure between the first and second determinations after the third hemorrhage.

However, an increase could hardly have been anticipated in view of the high values observed in the first determinations. In the two dogs subjected to the fourth bleeding, a value noticeably above normal, 26.6 millimeters, but lower than the value observed after the third hemorrhage, was noted. This value was based on two determinations for each animal.

Fibrinogen.—It is evident from values obtained that fibrinogen lost through hemorrhage is replaced rapidly. It may be noted from table 49 that the normal fibrinogen level was 0.26 g. per 100 cc. of plasma. The means observed following the first, second, third, and fourth hemorrhages were respectively 0.24, 0.26, 0.29, and 0.29 g. per 100 cc. of plasma.

DISCUSSION

Plasma Volume

Robertson and Bock's (1919) observations on wounded soldiers showed that the blood volume was restored relatively slowly after hemorrhage. This condition, they suggested, might be due to an insufficient quantity of reserve fluids in the tissue since, if the patients were given large quantities of fluid by alimentary tract, rapid increases in blood volume resulted and were maintained. This need for fluids was substantiated by the fact that the animals used in the present investigation evidenced thirst immediately after hemorrhage.

Ludwig (1931) postulated the existence of "plasma depots" similar to the "cell depots" which have been reported as existing in the body. He suggested the liver as the primary source of this reserve plasma. That a certain amount of reserve plasma exists in the body is apparent from the alterations noted in cell volume per cent within 30 minutes of the start of hemorrhage (during the short-term study) and before the animals had been permitted to ingest any water. Restoration of plasma volume was usually complete, and in most cases actually above the normal level, within 24 hours after bleeding.

The animals subjected to two bleedings, in the long-term study, in most instances showed plasma volumes appreciably above the normal level before the end of the first post-bleeding week.

Blood Plasma Constituents

The rapid recovery in the osmotic pressure of the blood, specifically the recovery in plasma proteins and the various calcium and phosphorus fractions, may partially account for the recovery in plasma volume. One factor that may contribute to the increase in the osmotic pressure of the blood is the nonprotein nitrogen. A number of investigators have reported increases in the nonprotein nitrogen level following hemorrhage

(Taylor and Lewis, 1915a; Kerr, Hurwitz, and Whipple, 1918-19; Buell, 1919; Schlutz, Swanson, and Ziegler, 1928). In general, the changes noted would appear to be transitory, lasting from only a few hours to two or three days.

In the short-term study, the nonprotein nitrogen level per unit of volume was slightly below normal during the post-bleeding period. However, the total amount in the circulating plasma after hemorrhage was in remarkable agreement with the observed pre-bleeding total. It is possible that the total amount in the circulation is of greater significance than the level in the blood. This is also true of the various calcium and phosphorus fractions (table 38).

Opinions differ as to the effect of hemorrhage on blood phosphorus. Malan (1928) found that repeated hemorrhage in sheep resulted in a lowering of total and lipide phosphorus, while the inorganic fraction showed no consistent variation. Contrary to these findings, Fishberg (1929) noted a rise in inorganic phosphorus following repeated bleeding of rabbits. Nitzescu and Runceanu (1927) reported that inorganic phosphorus rose slowly in dogs, and only in the last stage of a fatal hemorrhage. Youngburg and Youngburg (1936), using rats as experimental animals, observed "no change or, possibly, a decrease in the phosphorus compounds" which they studied—inorganic, organic acid soluble, and lipide phosphorus.

In the short-term study, values slightly below normal were observed for both lipide and inorganic phosphorus throughout the post-bleeding period. On the basis of the total amount in the circulating plasma, however, (table 38) the post-bleeding means exceeded slightly the pre-bleeding means, again suggesting, as in the case of protein and nonprotein nitrogen, that the total amount in the circulation is more significant than the level.

There was also a divergence of opinion regarding the effect of hemorrhage on the calcium level. Kauftheil and Kisch (1927b), working with rabbits rendered anemic through bleeding, found that blood calcium varied only slightly with the degree of anemia. On the other hand, Fishberg (1929), reporting a similar experiment on rabbits, noted a fall in serum calcium in severe hemorrhagic anemia. Sekitoo (1929-30) stated that repeated bleeding caused a slight hypocalcemia which, however, disappeared soon after the bleeding stopped, the calcium level returning quickly to normal. Kapsinow and Underhill (1929) noted a similar fall in blood calcium, owing to bleeding; Charles (1931-32) reported a rise; and Culhane (1930) and Mirvish and Bosman (1927-28) found no effect on the calcium level. Nitzescu and Runceanu (1927) observed in dogs and rabbits a marked rise in calcium immediately before death resulting from bleeding. Kauftheil and Kisch (1927a) reported that human blood calcium is almost always below normal in anemia.

In the short-term study, values that were slightly below normal were observed following hemorrhage for the diffusible calcium whereas non-diffusible calcium, 24 hours after bleeding, showed a mean level slightly above normal, values slightly below the normal being observed thereafter. As in the case of the phosphorus fractions, the total amount of the calcium fractions in the circulating plasma displayed, during the post-bleeding period, mean values that were somewhat above the pre-bleeding means, again suggesting the greater importance of the total quantity in the circulation than of the level.

Although some investigators (Peters and Eiserson, 1929, and Greenwald, 1931, working with human subjects, and Darrow and Cary, 1934, working with dogs) have reported relationships between protein and calcium of the plasma, no significant correlations were obtained in the present investigation, which was in accord with the report of Palmer, Gortner, and Rude (1930) covering their work with cattle.

There have been a number of excellent studies on the regeneration of plasma proteins following depletion. In some instances, the studies were based on hemorrhages in which a measured amount of the total blood volume was removed. In others, the plasmapheresis technique was employed. In this latter type of experiment, plasma protein regeneration was followed over a considerable period of time, using a variety of test foods. On the basis of these studies, potency ratios for various proteins were established (Pommerenke, Slavin, Kariher, and Whipple, 1935; Holman, Mahoney, and Whipple, 1934; McNaught, Scott, Woods, and Whipple, 1936; Madden, Winslow, Howland, and Whipple, 1937; Melnick, Cowgill, and Burack, 1936; and Melnick and Cowgill, 1937).

In the current research, in which plasma protein regeneration was observed in animals subjected to repeated bleedings, recovery of the albumin fraction occurred with great rapidity whereas globulin recovery was accomplished with difficulty. This observation agreed with those reported by Morawitz (1906) and Cuvelier and Patoir (1932), but not with those of Kerr, Hurwitz, and Whipple (1918-19) and Schlutz, Swanson, and Ziegler (1928), who reported a more rapid recovery of the globulin fraction following hemorrhage. Since Pommerenke, Slavin, Kariher, and Whipple have reported that plant proteins apparently favor the production of globulin, while animal proteins favor the regeneration of albumin, it is possible that the differences reported were dependent upon the experimental diet employed. In the present investigation, casein was used as the sole protein in the diet, which should and did favor the regeneration of albumin.

Whipple, Smith, and Belt (1920) postulated that the liver was involved in the regeneration of new plasma protein but suggested at the same time that other organs might be able in an emergency to function also in this capacity. Based on *in vitro* experiments, Bellis and Scott

(1935) reported that a considerable part of the plasma protein was derived from proteins adsorbed on the red corpuscles, released after hemorrhage through dilution of the plasma. According to these investigators, the increase in protein following dilution was due chiefly to albumin, although euglobulin (fibrinogen in oxalated blood) and pseudoglobulin I also were increased to some extent.

Apparently there was agreement among workers concerning the celerity with which the level of fibrinogen is restored in the plasma. Whipple, Smith, and Belt found that replacement of this protein occurred rapidly, the normal level being regained within 24 hours after hemorrhage. This confirmed the earlier work of Goodpasture (1914), who after having reduced the fibrinogen in the blood to a level where its presence could not be detected noted appreciable increases in this protein fraction within 15 minutes, followed within another 15 minutes by sufficient clotting to permit inversion of the tube. In the present investigation, in the animals that were bled three to four times, it was also observed that fibrinogen regeneration occurred very quickly. In general, the post-bleeding values were quite similar to the pre-bleeding normal levels.

SUMMARY

Plasma Volume

In the short-term study, within 30 minutes of the start of hemorrhage, in which 20 per cent of the total blood volume was removed, plasma volume had recovered to within 13 per cent of the pre-bleeding mean. Twenty-four hours later it had recovered to a level slightly above the pre-bleeding mean. On succeeding days further slight increases occurred to compensate for losses in cell volume owing to sampling. In the long-term study, for the females plasma volume per kilogram was appreciably above the pre-bleeding mean one week after hemorrhage, with gradual decreases thereafter to a level approaching the pre-bleeding normal six weeks after hemorrhage. For the males, plasma volume per kilogram was also considerably above the pre-bleeding mean one week after hemorrhage. Thereafter gradual decreases resulted in a mean slightly above the pre-bleeding level six weeks after hemorrhage. Little alteration was noted for either males or females throughout the remainder of the post-bleeding period.

Blood Plasma Constituents

In the short-term study, both the protein nitrogen and nonprotein nitrogen levels were somewhat below the pre-bleeding means throughout the post-bleeding period. The mean of the total protein nitrogen in the circulating plasma after hemorrhage was slightly greater than the pre-

bleeding mean, while the mean of the total amount of nonprotein nitrogen after hemorrhage was identical with the pre-bleeding value.

The lipide and inorganic phosphorus levels were somewhat below the pre-bleeding means throughout the post-bleeding period, which condition was reflected in the total phosphorus level. For both phosphorus fractions, the mean total amount in the circulating plasma after hemorrhage was slightly greater than the pre-bleeding mean.

The level of diffusible calcium was slightly below the pre-bleeding mean throughout the post-bleeding period. For nondiffusible calcium, the mean level 24 hours after bleeding was somewhat higher than the pre-bleeding mean, but values below the pre-bleeding mean were observed throughout the remainder of the post-bleeding period. The mean total amount of the calcium fractions in the circulating plasma after hemorrhage was slightly greater than the pre-bleeding mean.

Significant positive correlations were obtained between the levels of plasma protein and inorganic phosphorus before hemorrhage, +.3248, and after hemorrhage, +.3487.

Significant positive correlations were found, after hemorrhage, between lipide phosphorus and nonprotein nitrogen levels, +.2575; between lipide phosphorus and inorganic phosphorus, +.4371; between lipide phosphorus and diffusible calcium, +.3077; and between inorganic phosphorus and diffusible calcium, +.3897. No such relationships were apparent in pre-bleeding levels.

A significant negative correlation was obtained between pre-bleeding levels for diffusible and nondiffusible calcium, —.3787, but not between post-bleeding values.

In the fourth study, in which the dogs were bled three or four times, the mean albumin level returned promptly to the pre-bleeding normal following each unit hemorrhage, attaining a level somewhat above normal after the third and fourth hemorrhages. The globulin level failed to return to the pre-bleeding normal after the first three unit hemorrhages, although apparently there was increasing ability to regenerate globulin as shown by increased means following each unit hemorrhage. Alterations in the levels of albumin and globulin were reflected in the albumin-globulin ratio. Calculation of the osmotic pressure contributed by the albumin and globulin showed a prompt return to the pre-bleeding normal, with values above normal after the third and fourth hemorrhages.

The fibrinogen level recovered promptly following each unit hemorrhage, the means obtained after the third and fourth hemorrhages being slightly above the pre-bleeding mean.

VI. WHOLE BLOOD

TOTAL BLOOD VOLUME

As POINTED OUT in section IV on blood cells, the failure of the total blood volume to return to normal following hemorrhage leads to an erroneous conception of the degree of anemia induced by bleeding when red cell count, oxygen-combining capacity, or cell volume per cent is used as the criterion for diagnosing the condition of an anemic patient.

The mean pre-bleeding total volume for the animals used in the short-term study was 75.5 cc. per kilogram (table 50). Within 30 minutes after the start of bleeding the total blood volume had decreased 17 per cent, to a mean of 62.9 cc. per kilogram. The rapid restoration of plasma explains why this decrease did not more nearly approximate the percentage of the blood removed. On the day following hemorrhage, the mean was 67.8 cc. per kilogram, followed by slightly lower values throughout the remainder of the post-bleeding period.

Table 50. Values for Total Blood Volume, in Cubic Centimeters per Kilogram (Short-term study)

Dog No.	Last pre-bleeding	Within 30 minutes of start of bleeding	Post-bleeding period			
			1 day	2 days	3 days	5-7 days
Males						
44	75.8	62.5	67.8	66.1	62.7	62.8
	74.6	56.8	65.9	65.5	63.7	67.7
45	78.4	.....	69.7	70.4	69.2	67.3
	77.5	58.5	73.5	65.0	66.5	65.7
46	81.3	74.0	77.1	72.6	76.4	71.0
	80.8	68.9	65.3	69.7	68.6	66.7
47	73.4	66.7	66.4	56.8	55.9	67.5
	74.3	65.2	63.4	67.2	67.4	68.1
	71.1	55.7	58.2	60.5	58.7	57.6
	67.8	57.8	70.1	62.6	66.5	65.6
Mean	75.5	62.9	67.8	65.6	65.6	66.0

In the long-term study the pre-bleeding mean for the females was 67.6 cc. per kilogram (table 51). One week following hemorrhage, the mean value was 59.0 cc., where it remained practically constant throughout the first six weeks of the post-bleeding period. It will be recalled that these animals made practically no progress in restoring total cell volume (table 20), which condition was reflected in the failure of the total blood volume to recover throughout this six-week interval. The mean value noted for the females observed for 12 weeks, 56.8 cc., was

almost identical with that observed for these animals at the end of the sixth week, 56.9 cc. per kilogram. Following the initial adjustment to blood loss by increase in plasma, further adjustment was evidently dependent upon the ability or inability of the animal to restore cell volume in that the animals that were most capable of cell volume regeneration

Table 51. Values for Total Blood Volume, in Cubic Centimeters per Kilogram (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-5 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	68.6	59.8	64.2	56.1	64.6	59.5	59.8	64.0	65.9
21	68.5	69.4	63.8	66.3	55.4	62.2	60.9	63.4	.....
22	61.8	59.4	50.7	58.3	54.6	57.1	61.0	60.7	.....
19	70.2	64.4	56.1	56.3	.....	59.7	52.6	50.8	47.1
32	69.0	56.6	60.1	57.6	57.1	59.0	57.1	56.4	57.5
Mean	67.6	61.9	59.0	58.9	57.9	59.5	58.3	59.1	56.8
Males									
17	69.8	59.1	64.0	64.6	69.1	61.7	58.5	65.4	70.6
18	58.4	54.9	50.0	48.5	56.8	60.3	54.2	53.2	56.1
19	75.4	72.0	73.3	78.1	78.8	75.4	85.6	80.0	74.8
31	71.4	62.0	69.3	70.6	70.6	72.0	75.3	71.1	70.6
Mean	68.8	62.0	64.1	65.4	68.8	67.4	68.4	67.4	68.0

also most nearly approached their normal total blood volume. This is further evidenced by the data secured on the males in this study in that these animals displayed a greater capacity for cell volume regeneration than did the females, and therefore also a greater recovery in total blood volume. The mean pre-bleeding value for the males was 68.8 cc. per kilogram. One week after hemorrhage, a value of 64.1 cc. was observed. Total blood volume recovered to the normal level by the third week and continued at practically the same level throughout the rest of the post-bleeding period.

CONSTITUENTS OF WHOLE BLOOD

Protein Nitrogen.—The data presented in this section were secured in the long-term study. The pre-bleeding mean value for protein nitrogen of whole blood for the five females used in this study was 3.08 g. per 100 cc. (table 52). One week after hemorrhage a value of 2.56 g. was obtained, with a further reduction to 2.44 g. at the end of the sixth post-bleeding week, representing 79 per cent of the normal. For the two females observed for 12 weeks, the protein nitrogen level was found to be 2.44 g., contrasted with a value of 2.19 g. observed for these animals

at the end of the sixth week, indicating a very slight increase during the second six-week portion of the post-bleeding period. Since approximately two thirds of the nitrogen of the blood is found in the red blood cells, the failure of the experimental animals to recover their cell volume

Table 52. Values for Total Protein Nitrogen in Blood, in Grams per 100 Cubic Centimeters (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	2.86	2.20	2.53	2.24	2.01	2.36	2.24	2.08	2.61
21	3.14	2.48	2.76	2.46	2.66	2.74	2.60	2.59	.....
22	2.81	2.27	2.30	2.44	2.52	2.56	2.69	2.67	.....
19	3.44	2.62	2.77	2.64	2.47	2.67	2.65	2.30	2.28
32	3.14	2.47	2.44	2.32	2.41	2.44	2.52	2.57	.....
Mean	3.08	2.41	2.56	2.42	2.41	2.55	2.54	2.44	2.44
Males									
17	3.33	2.52	2.44	2.84	2.66	2.95	2.74	2.76	2.72
18	3.39	2.41	2.28	2.24	2.34	2.30	2.40	2.26	2.34
30	2.94	2.36	2.12	2.22	2.82	2.94	3.05	2.79	.....
31	3.32	2.58	2.05	2.71	2.80	2.89	2.95	2.96	.....
Mean	3.24	2.47	2.22	2.50	2.66	2.77	2.78	2.69	2.53

after hemorrhage readily explains the continued low total protein nitrogen in the blood.

The male dogs, it will be recalled, made considerably better progress than the females in recovering their normal cell volume, which tendency was again evidenced by their greater recovery in total protein nitrogen. The pre-bleeding normal level of total protein nitrogen was 3.24 g. per 100 cubic centimeters. At the end of the first week after hemorrhage a value of 2.22 g. per 100 cc. was obtained; at the end of the sixth week, 2.69 g., the latter value representing 83 per cent of the pre-bleeding normal. By the end of the twelfth week, the total protein nitrogen level, based on two dogs, was 2.53 g. per 100 cc., showing no change from the mean noted for these two animals at the end of the sixth week, 2.51 per 100 cubic centimeters.

Nonprotein Nitrogen.—The mean pre-bleeding nonprotein nitrogen level for both male and female animals was 32.4 mg. per 100 cc. of blood (table 53). At the end of the first post-bleeding week, the mean for the females was 36.6 milligrams; for the males, 33.2 milligrams; with values of 34.3 mg. and 31.0 mg. for the females and male, respectively, at the end of the sixth post-bleeding week. For the two females observed for 12 weeks, the level noted at the end of the period was 30.0 mg., contrasted with 31.8 mg. for these same animals at the end of the sixth

week. For the males, the value (based on two animals) observed at the end of the twelfth week was 26.4 mg., which value was in close agreement with the mean for these same animals at the end of the sixth week, 27.0 milligrams.

Table 53. Values for Nonprotein Nitrogen in Blood, in Milligrams per 100 Cubic Centimeters (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
20	28.0	35.1	32.5	34.5	28.3	31.1	27.9	28.5	31.5
21	25.5	34.7	32.5	27.7	37.1	29.7	26.0	32.1	.....
22	38.9	53.3	51.5	39.5	34.5	37.9	46.9	41.5	.....
19	37.3	32.6	35.6	33.0	31.2	38.0	31.0	35.2	28.6
32	32.4	35.8	30.9	32.7	32.0	34.4	30.0	34.4	.....
Mean	32.4	38.1	36.6	33.5	32.6	34.2	32.4	34.3	30.0
Males									
17	30.1	26.7	24.2	26.8	27.6	26.3	27.2	25.8	29.1
18	29.9	28.0	30.2	29.1	28.9	27.1	28.8	28.2	23.8
30	35.1	32.5	39.1	38.3	35.8	34.2	32.8	32.0	.....
31	34.6	35.6	39.1	38.3	37.4	35.0	35.6	37.8	.....
Mean	32.4	30.7	33.2	33.1	32.4	30.6	31.1	31.0	26.4

Urea Nitrogen.—Two of the females and four of the males were used for urea nitrogen determinations on whole blood. For the females, the pre-bleeding normal level was found to be 12.7 mg. per 100 cc., for the males, 11.0 mg. (table 54). Following hemorrhage, the level was distinctly above normal for both sexes, reaching a mean of 17.4 mg. for the females, 17.7 mg. for the males, at the end of the first post-bleeding week. Both females showed high post-bleeding urea nitrogen values, whereas the high post-bleeding mean for the males was the result of marked increases in three of the dogs, with comparatively little change in the fourth animal. In four of the five animals showing a post-bleeding rise in urea nitrogen, there was a decline toward the normal level throughout the post-bleeding period. The fifth animal, however, continued to show very high urea nitrogen levels. By the end of the twelfth post-bleeding week the urea nitrogen levels of the three animals that were observed for this length of time (one female and two males) had returned to normal.

Amino Acid Nitrogen and Creatinine.—Data on amino acid nitrogen (table 55) and creatinine (table 56), in milligrams per 100 cc. of blood, were obtained on one female and two males. Although the post-bleeding level of amino acid nitrogen appeared to be distinctly higher than

Table 54. Values for Urea Nitrogen in Blood, in Milligrams per 100 Cubic Centimeters (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Females									
19	18.02	18.61	25.07	20.39	14.83	18.80	16.94	18.80	13.21
32	7.40	17.89	9.75	19.61	22.46	.....	16.86	11.27	.....
Mean	12.71	18.25	17.41	20.00	18.64	18.80	16.90	15.03	13.21
Males									
17	13.74	14.23	11.00	13.71	14.72	9.11	11.52	10.86	11.47
18	13.79	15.36	17.17	17.35	14.83	12.82	14.21	12.77	6.89
30	8.88	6.82	17.78	25.53	21.33	21.55	21.88	.....	.....
31	7.68	20.51	25.02	16.55	30.65	23.99	.....	31.91	.....
Mean	11.02	14.23	17.74	18.28	20.38	16.87	15.87	18.51	9.18

Table 55. Values for Amino Acid Nitrogen in Blood, in Milligrams per 100 Cubic Centimeters (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Female									
19	4.05	16.73	17.27	18.10	21.12	16.25	17.11	7.08	16.65
Males									
17	4.84	7.34	14.62	15.92	12.27	7.52	9.73	12.97	8.19
18	.....	.....	3.61	8.74	10.56	12.86	13.11	20.98	10.42
Mean	4.84	7.34	9.11	12.33	11.41	10.19	11.42	16.97	9.30

Table 56. Values for Creatinine in Blood, in Milligrams per 100 Cubic Centimeters (Long-term study)

Dog No.	Pre-bleeding normal	Post-bleeding period							
		1-2 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	12 wks.
Female									
19	1.72	1.65	1.63	1.66	1.58	1.55	1.56	1.38	1.52
Males									
17	1.44	1.57	1.61	1.58	1.58	1.55	1.58	1.56	1.52
18	1.81	1.65	1.74	1.63	1.70	1.70	1.66	1.70	1.70
Mean	1.62	1.61	1.67	1.60	1.64	1.62	1.62	1.63	1.61

the normal level, such sharp fluctuations occurred at times during the post-bleeding period that because of the limited number of normal values secured it cannot be said positively that such a condition prevailed.

The level for creatinine remained surprisingly constant in the two male animals throughout the post-bleeding period, whereas a slight decrease was noted for the female.

DISCUSSION

Total Blood Volume

In the present research, it was evident that following hemorrhage the animals could adapt themselves to a total blood volume which was appreciably less than normal. Although some restoration of total blood volume occurred, owing to the increase in plasma, complete restoration was limited by recovery in cell volume. In the short-term study, the total blood volume 30 minutes after the start of bleeding was found to be 17 per cent below the pre-bleeding mean, followed 24 hours later by recovery to a level which was only 10 per cent below, and on the third day by a value which was only 13 per cent below the pre-bleeding mean. In the long-term study, the female animals displayed total blood volumes 13 per cent below normal even six weeks after bleeding. The total blood volume of the males, however, remained below normal only two weeks after bleeding. Thereafter their total blood volume remained normal, owing to their more satisfactory recovery in cell volume.

The observations in the present study confirmed the results reported by Robertson and Bock (1919) from their work with wounded soldiers, in whom they observed marked reductions in total blood volume following hemorrhage. They noted increases in blood volume coincident with the rapid increase in the percentage of reticulated cells, or with the addition of new cells to the circulation through transfusion.

Constituents of Whole Blood

Little information was available in the literature concerning variations after hemorrhage in the protein nitrogen of the blood. Hawk and Gies (1904), working with dogs, and Buell (1919), experimenting on pigs, reported a decrease following bleeding in both the total and protein nitrogen.

In the long-term study, recovery in protein nitrogen of the whole blood was retarded by the slow recovery in the volume of red blood cells. As pointed out in the section on plasma constituents, recovery in plasma protein nitrogen, particularly that of the albumin and fibrinogen, was prompt and complete within a few days after hemorrhage.

Increases in the nonprotein nitrogen level of the blood following

hemorrhage have been reported by a number of workers, which results are in accord with the findings in the present investigation, particularly as concerns the females, who showed increases following bleeding, with gradual return to normal during the post-bleeding period.

Increases in blood urea nitrogen would appear to account for a large portion of the increases noted in nonprotein nitrogen following hemorrhage, as shown by the work of Taylor and Lewis (1915), who as the result of observations on one dog reported a marked increase in urea nitrogen following repeated hemorrhage. Buell (1919) noted slight increases after bleeding in urea nitrogen of the blood of pigs, the increases occurring at intervals which varied in duration. An increase in blood urea was also reported by Dubois and Polonovski (1924) as existing after bleeding in both rabbits and dogs. On the other hand, Brocq-Rousseu, Roussel, and Gallot (1928), analyzing the blood of 36 horses at the beginning and at the end of a massive hemorrhage, found urea nitrogen of the blood to be practically unchanged. Dumas and Pagès (1930) obtained similar results on their three subjects.

In human subjects, Borst (1936) and Wood (1936) found a rather definite rise in blood urea following severe gastric hemorrhage. The former attributed the condition to an increased urea formation owing to decomposition and resorption of exuded blood, while the latter believed it might be due to a decreased kidney function as a result of oxygen-want following great loss of hemoglobin. He found that when the hemoglobin level was brought up rather rapidly to a fairly normal level by use of a continuous massive transfusion of citrated blood, the blood urea level promptly fell to within normal limits.

In the present investigation, increases in urea nitrogen of the blood were noted following hemorrhage in five of the six animals on which the determination was made. No relationship was apparent between the oxygen-combining capacity of the animals and the level of urea nitrogen. Therefore, observations on these animals were not in accord with Wood's suggestion that increases in urea nitrogen follow kidney failure due to lowered hemoglobin content of the blood.

A number of research workers have reported on the effects of hemorrhage upon the amino acid content of the blood. György and Zunz (1915) stated that although the amino acid content of the blood of dogs remained remarkably constant under normal conditions copious bleeding resulted in a slight increase, although when Ringer's solution was injected simultaneously the amino acid content tended to decrease. This latter observation is probably due to the effect of dilution of the blood with Ringer's solution. Taylor and Lewis (1915a), in a similar experiment, observed a rise in the amino acid nitrogen of the blood of one dog, which they attributed to the release of stored amino acids, or amino acids derived from the hydrolysis of tissue or serum proteins. Tochowicz (1936-37) observed normal levels of amino acids in both the whole blood and plasma in patients with secondary anemia.

In the current study, there seemed to be a rise in the amino acid nitrogen of the blood following bleeding. The limited number of observations made, however, (on three dogs) does not warrant too definite conclusions on this point.

No data were found in the literature covering the effect of hemorrhage on the creatinine level. In this research, there appeared to be a decrease in the creatinine content of the blood of the one female on which the determination was made, whereas in the two male animals no alteration was noted.

SUMMARY

Total Blood Volume

In the short-term study, a 17 per cent decrease in blood volume was observed 30 minutes after the start of bleeding, followed 24 hours later by recovery to a level which was only 10 per cent below the pre-bleeding mean, with slightly lower values throughout the balance of the post-bleeding period. In the long-term study, the females showed a 13 per cent decrease as a result of hemorrhage at the end of the first post-bleeding week, and no recovery during the balance of the post-bleeding period. The males showed a 7 per cent decrease at the end of the first post-bleeding week, followed by recovery to the pre-bleeding level early in the post-bleeding period.

Constituents of Whole Blood

In the long-term study, for the females the protein nitrogen remained distinctly below the pre-bleeding mean during the post-bleeding period and reflected their failure to restore cell volume. Similarly for the males, although some recovery was noted, the protein nitrogen level remained distinctly below the pre-bleeding mean throughout the post-bleeding period. For both males and females the mean nonprotein nitrogen level fluctuated throughout the post-bleeding period, the females showing slightly higher means.

In the urea nitrogen levels, fluctuations were observed for both males and females during the post-bleeding period, the values in general being above the pre-bleeding means.

There were sharp fluctuations in the amino acid nitrogen level throughout the post-bleeding period, with means higher than pre-bleeding values.

The mean pre-bleeding creatinine level was maintained throughout the post-bleeding period by the two males, whereas the one female showed a slight decrease.

VII. GENERAL DISCUSSION

HEMORRHAGIC and hypochromic anemias occur often enough and are of sufficient human significance to be included in the classifications of anemias proposed by authorities and presented by Downey (1938) in a recent comprehensive resumé of the field. Wintrobe and Beebe (1933) listed 15 different names that had been used and that were apparently considered as synonymous terms applicable to the condition described by them as idiopathic hypochromic anemia. The use of the term "idiopathic" indicates uncertainty as to the causes of this condition. "Hypochromic" is associated with a deficiency of the pigment, hemoglobin; "microcytic," a term proposed by other workers (Witts, 1931; Vanderhoof and Davis, 1932; and Wintrobe, 1934), is applied to undersized erythrocytes.

Scattered statements in the literature support the theory that hemorrhages in different parts of the body may cause hypochromic anemia. It occurs much more frequently in women, especially between the ages of 20 and 50 years, than in men (Witts, 1931). Fullerton (1936) claimed that the loss of iron during the reproductive period of women was of great importance and that cumulative losses due to menstruation, pregnancy, and lactation resulted in a gradually falling hemoglobin level until the menopause, after which a marked rise was noted. Strauss and Castle (1933) regarded the development of blood in the fetus as equivalent to a blood loss in the mother and stated that repeated pregnancies have resulted in marked hypochromia. Others cited periodic menstrual losses as possible causes (Witts, 1931; Wintrobe and Beebe, 1933; Wright, 1933; Barer, Fowler, and Baldridge, 1934-35; and Fowler and Barer, 1937). On the other hand, Haden and Singleton (1933) did not regard excessive menstrual hemorrhages alone as responsible for anemia since in their series of cases menstrual disorders were usually relieved when the blood picture returned to normal. Vanderhoof and Davis (1932) did not consider periodic blood losses as important for the five women whom they studied.

The similarity between the blood picture observed following acute or recurrent blood losses and that noted in hypochromic anemia has been emphasized (Heath, 1933; Castle and Minot, 1936; and Fowler and Barer, 1937). Witts (1931) stated that the occurrence of this type of anemia in men was indicative of blood loss, the source of which should be located.

The blood pictures cited for hypochromic or microcytic anemias are paralleled by those reported for hemorrhagic anemia as based on the results of this investigation. Hemoglobin, whether measured in terms

of the level, or of the color index, or of mean corpuscular hemoglobin concentration, has always been found to be low in hypochromic anemia (Wintrobe and Beebe, 1933; Haden and Singleton, 1933; Wintrobe, 1934; Sturgis, 1938; McLean, 1938; Fowler and Barer, 1939). In the experimental animals used in the current studies hemoglobin failed to return to normal at any time during the course of the research. Red cell counts have been reported as reduced in number (Wintrobe, 1934; Castle and Minot, 1936), normal or relatively high (Wintrobe and Beebe, 1933; Haden and Singleton, 1933), or increased when paralleled by microcytosis (Sturgis, 1938). In the experimental animals red cell counts were low in the early stages of recovery, but increased rapidly in number, reaching ultimately a level considerably above normal. Most investigators who have studied cell size have reported alterations ranging from slight to marked decreases (Wintrobe, 1929-30; Price-Jones, 1932; Wintrobe and Beebe, 1933; Wintrobe, 1934; Castle and Minot, 1936; Sturgis, 1938; Bethell, Isaacs, Goldhamer, and Sturgis, 1938; and Fowler and Barer, 1939). In the present research, decreases in cell size were noted invariably, the extent of decrease varying with the blood losses to which the animals were subjected.

Thus similarities noted in the blood pictures of the experimental animals in which hemorrhagic anemia had been induced, and of human subjects afflicted with idiopathic hypochromic anemia, substantiate the hypothesis that the latter type is frequently the result of hemorrhage. These studies do not, of course, preclude the existence of other causes of hypochromic anemia.

To secure a comprehensive picture of blood changes occurring in hemorrhagic anemia, the dogs used in these experiments were maintained under controlled conditions and subjected to bleedings of such magnitude as to simulate hemorrhages occurring in human subjects such as those involved in accidents, operations, childbirth, menorrhagia, and the like.

It must be appreciated that these same controlled conditions made it possible to secure a more accurate picture of alterations in the blood caused by hemorrhage than would have been possible with human subjects, since the animals selected were normal healthy dogs, maintained on a balanced diet and subjected to bleedings regulated in size and frequency, under circumstances that were not complicated by other pathological or physiological influences.

Observation showed that at progressive stages in recovery certain combinations of blood measures were more satisfactory criteria than others, the combination varying from time to time. Therefore, it is essential that the changing significance of these measures be kept in mind in assessing the status of individuals who have experienced blood losses and in following the progress of recovery in anemic persons.

Hemorrhage results in the loss of both plasma and cells. In this investigation, immediately after acute bleeding, the hematocrit, the red cell count, and the levels of constituents such as hemoglobin showed

little change and gave no indication of the extent of the hemorrhage. Shortly thereafter dilution with interstitial fluid occurred bringing about a rapid restoration of plasma volume, with the result that hematocrits, red cell counts, and hemoglobin decreased simultaneously. The replacement of erythrocytes was a slow process which in these experiments was not complete even after many weeks. Marked shifts in these measurements are therefore characteristic of the adjustments taking place soon after a severe hemorrhage and are consequently significant in establishing or verifying the occurrence, the recency, or the extent of hemorrhage.

Certain measures are of infinitely greater significance than others as a means of establishing the status of a person following blood loss. Thus judgment of an individual's condition, based on the hemoglobin level, red cell count, or red cell hematocrit, in the very early stages of recovery would give an erroneous conception and create a false sense of security. It is therefore evident that to secure an adequate basis for assessment it is necessary to secure total plasma volume and total cell volume data to be used in conjunction with other measures. In the later stages of recovery also, red cell counts are of limited usefulness, since an increase in count may be associated with a decrease in cell size, resulting in a total cell volume still markedly below the normal level. For example, a high count, associated with small size, suggests repeated or long-continued blood losses; a low red cell count, with cells of normal size, is indicative of a recent severe hemorrhage. It may be concluded, therefore, that several carefully selected combinations of blood measurements will more adequately evaluate the condition of a person after bleeding has taken place than any single criterion.

The current research, which covered a considerable period of time, demonstrated that certain factors returned to normal rapidly, whereas others (hemoglobin, red cell volume, and red cell size) returned to normal slowly, in some instances not at all, and therefore needed special consideration. The latter correspond to those factors which have been shown clinically to be subnormal in human subjects suffering from the types of anemia under discussion. This investigation has established the relative merits of different measures of recovery.

The synthetic diet used in the present experiments permitted limited recovery in cell volume and hemoglobin. It was evident that the diet, although quite adequate for maintaining animals in a normal condition over long periods of time, was of limited usefulness in restoring the blood following bleeding. Although the iron content was fairly generous, it is possible that a higher iron intake would have resulted in more rapid regeneration. Other undetermined factors may have played a role also in limiting recovery.

Hypochromic anemia caused by blood losses constitutes a real nutritional problem. It is obvious that the characteristics of hemorrhagic anemia observed in these studies closely resemble those which have been

reported by other observers as characteristic of hypochromic microcytic anemia in humans. The advantages of conducting studies like those reported in this bulletin are self-evident. The normal blood picture of dogs has been portrayed, thus affording a basis for selecting suitable animals for the experiments and simultaneously establishing goals of achievement for corrective nutritional programs. Procedures employing hemorrhages have been evolved and described, by the use of which hypochromic microcytic anemias of varying degrees of severity may be produced. Blood pictures characteristic of different phases of the post-hemorrhagic period have been set forth and the factors which return to normal with difficulty have been located.

The techniques described, the normals established, and the location of the factors which improve slowly, make it possible to produce in animals an hypochromic microcytic anemia associated with blood losses, and to evaluate dietary regimes designed to restore the blood picture to normal.

VIII. SUMMARY

1. This bulletin presents a comprehensive description of changes occurring in the blood pictures of 19 male and female dogs maintained on what is believed to be an adequate synthetic diet for adult dogs and subjected to massive bleedings, after which they were observed for periods varying in length.

2. Detailed summaries of the findings are presented at the end of each section—normal blood constituents, page 32; erythrocytes and leucocytes, pages 60 to 63; blood plasma, pages 78 to 79; and whole blood, page 87.

3. Within 30 minutes after hemorrhage, the most outstanding blood changes noted are the decrease in total blood volume and the drop in cell volume per cent, paralleling an immediate increase in plasma volume and slightly enlarged erythrocytes. At this time, dilution with interstitial fluid is not sufficient to modify very much the levels of the several blood constituents.

4. By the end of the first week, a typical blood picture of hemorrhagic anemia of short duration is obtained. The erythrocyte volume is distinctly below normal, the plasma volume exceeds somewhat the pre-bleeding level, while the total blood volume is still below normal. Serum, albumin, and fibrinogen levels are restored to normal, whereas total nitrogen in the blood, hemoglobin, serum globulin, and nonprotein nitrogen are below the pre-bleeding means. Hypochromia is observed. The levels of the plasma calcium and phosphorus fractions are below

normal, but the totals for both exceed the amounts originally present in the plasma. No significant leucocytosis is noted. Red cell counts are below normal and the size of the cells is decreased.

5. In the animals observed 6 to 12 weeks, a blood picture typical of hemorrhagic anemia of long standing is found. Both total blood volume and plasma volume approach pre-bleeding levels, but the total erythrocyte volume is still below normal. Relatively little recovery is noted in hemoglobin and total protein in the blood. The erythrocyte counts may be normal or above normal, but the individual cell volume or diameter is decreased, a relationship reflected in the significant negative correlation between these variables. Thus both hypochromia and microcytosis are present. In many cases, the progress observed in the males is greater than that noted in the females.

6. No single measurement adequately assesses the status of an individual after the loss of significant amounts of blood. The most serious blood changes involve the modified erythrocyte picture—total cell volume, cell volume per cent, erythrocyte count in relation to individual cell volume and diameter, and hemoglobin content of the cells.

7. The rate of erythrocyte regeneration depends upon the degree of physiological stress caused by hemorrhage, upon the reserves of cell-building materials in the animal body, and to a limited extent, upon the amount of food consumed.

8. Statistically significant relationships were observed between some of the blood constituents before bleeding, in others after bleeding.

9. Animals given a diet that will maintain a normal blood picture under ordinary conditions develop an anemia characterized by hypochromia and microcytosis when subjected to severe or recurrent hemorrhages.

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APPENDIX

Table I. Physical and Chemical Measurements on

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns
1	Pre-hemorrhage	7 days	75	74.2	34.1	45.9	7.33	62.6
2		6 days	74	77.5	33.0	42.6	7.01	60.8
3		4 days	83	68.1	28.5	41.9	7.16	58.6
4		2 days	69	67.5	26.8	39.7	7.04	56.4
5		1 day	28	75.8	29.2	38.6	.....	.....
6		same day	10	.....	.....	.....	.....	.....
7	First Hemorrhage		301	.....	.....	.....	.....	.....
8	Post-hemorrhage	30 min.	55	62.5	20.6	33.0	5.48	60.2
9		1 day	84	67.8	21.1	31.2	5.24	59.6
10		2 days	73	66.1	18.6	28.4	5.12	55.5
11		3 days	70	62.7	16.3	26.0	4.64	56.0
12		5 days	71	62.8	16.8	26.8	4.83	55.5
13		6 days	72	62.1	13.9	22.4	3.90	57.4
14		14 days	70	68.0	21.3	31.4	4.78	65.6
15	Pre-hemorrhage	13 days	76	.....	.....	.....	6.74	.....
16		12 days	75	70.1	27.0	38.6	6.74	57.3
17		11 days	71	74.2	27.4	36.9	6.54	56.5
18		10 days	72	74.6	26.4	35.2	6.26	56.2
19		same day	12	.....	.....	.....	.....	.....
20	Second Hemorrhage		313	.....	.....	.....	.....	.....
21	Post-hemorrhage	30 min.	63	56.8	19.6	34.5	5.54	62.2
22		1 day	75	65.9	20.5	31.2	5.10	61.1
23		2 days	72	65.5	20.1	30.7	4.68	65.6
24		3 days	73	63.7	17.1	26.9	4.56	58.9
25		4 days	74	67.7	19.4	28.7	4.96	57.8
26		6 days	74	65.5	16.6	25.3	4.16	60.9
27		23 days	77	64.1	20.9	32.7	5.42	60.3
28		24 days	78	70.3	22.3	31.8	5.08	62.7

Dog No. 44 ♂ (Mean Weight, 22.9 Kilograms)

Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in plasma, g. per 100 cc.	Protein nitrogen in plasma, g. per 100 cc.	Total phosphorus in plasma, mg. per 100 cc.	Lipide phosphorus in plasma, mg. per 100 cc.	Inorganic phosphorus in plasma, mg. per 100 cc.	Diffusible calcium in plasma, mg. per 100 cc.	Non-diffusible calcium in plasma, mg. per 100 cc.	
16.5	7.41	0.026	1.111	26.0	17.2	5.75	8.17	5.34	1
15.8	6.09	0.023	1.048	25.8	17.1	6.27	8.07	5.65	2
16.6	8.38	0.022	1.053	26.1	17.3	5.83	8.05	4.94	3
15.6	7.20	0.020	1.062	26.4	19.5	5.54	8.37	4.62	4
.....	.....	0.021	1.040	.....	.....	.....	.....	.....	5
.....	.....	.....	.....	26.8	18.9	6.49	8.82	5.44	6
.....	.....	.....	.....	.....	.....	.....	.....	.....	7
13.9	5.08	0.022	0.986	24.7	18.6	6.01	8.16	5.93	8
12.0	6.24	0.020	1.049	23.9	18.4	5.79	6.82	6.83	9
10.9	6.64	0.019	1.001	25.0	18.8	6.53	7.66	.....	10
10.8	6.01	0.019	0.978	23.8	16.6	5.67	8.26	3.86	11
10.7	6.54	0.019	1.003	23.8	17.2	5.96	8.57	4.73	12
9.8	5.99	0.020	0.959	24.2	19.0	5.90	7.83	6.12	13
12.3	10.16	0.023	1.099	25.2	15.9	7.02	8.02	4.23	14
15.2	8.04	0.021	1.094	26.9	18.2	6.99	7.64	4.36	15
14.6	12.46	0.020	1.119	22.6	16.5	5.26	8.48	4.89	16
15.5	9.52	0.021	1.119	24.6	17.5	5.32	7.38	4.24	17
14.0	8.89	0.019	1.085	24.6	17.8	5.29	7.31	4.20	18
.....	.....	.....	.....	23.5	17.8	5.76	6.29	4.80	19
.....	.....	.....	.....	.....	.....	.....	.....	.....	20
14.8	5.90	0.020	1.060	22.5	15.5	5.36	6.03	5.42	21
13.2	6.99	0.018	1.025	24.9	17.6	6.13	6.27	4.90	22
12.2	7.45	0.018	1.026	23.8	17.9	5.93	6.11	6.37	23
10.7	6.21	0.017	0.979	22.5	15.0	5.43	5.98	5.47	24
11.3	6.21	0.018	0.981	22.4	16.7	5.62	6.29	3.80	25
9.8	5.55	0.018	0.993	22.3	16.6	5.36	6.19	4.52	26
12.4	6.42	0.022	1.111	24.2	17.9	6.53	6.89	6.17	27
11.9	8.29	0.023	1.116	22.9	18.7	5.45	6.28	6.32	28

Table II. Physical and Chemical Measurements on

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns
1	Pre-hemorrhage	35 days	63	83.2	44.4	53.4	7.11	75.2
2		33 days	65	79.0	40.2	50.9	7.85	64.8
3		30 days	65	80.1	41.1	51.4	7.06	72.9
4		28 days	65	.....	.....	.....	7.94	.....
5		27 days	63	72.4	37.2	51.4	7.00	73.5
6		9 days	68	81.9	43.8	53.5	7.62	70.2
7		6 days	72	77.6	39.9	51.8	7.98	64.9
8		5 days	73	84.4	44.9	53.3	7.86	67.8
9		2 days	73	78.4	38.6	49.3	7.84	62.9
10		same day	12	.....	.....	.....	.....	.....
11	First Hemorrhage		273	.....	.....	.....	.....	.....
12	Post-hemorrhage	30 min.	53	.....	.....	.....	7.06	.....
13		1 day	63	69.7	25.7	36.9	6.48	56.9
14		2 days	68	70.4	25.9	36.8	5.94	61.9
15		3 days	67	69.2	23.5	33.9	5.93	57.2
16		5 days	66	67.3	22.7	33.8	5.42	62.3
17		6 days	68	66.8	23.1	34.6	5.86	59.0
18		8 days	68	68.2	21.9	32.1	5.17	62.1
19	Pre-hemorrhage	7 days	72	73.5	37.4	51.0	8.34	61.2
20		6 days	74	74.7	36.5	48.9	8.20	59.7
21		5 days	75	77.7	37.7	48.5	8.15	59.5
22		4 days	75	77.5	36.0	46.5	8.04	57.8
23		same day	12	.....	.....	.....	.....	.....
24	Second Hemorrhage		250	.....	.....	.....	.....	.....
25	Post-hemorrhage	30 min.	63	58.5	25.5	43.8	7.27	60.2
26		1 day	70	73.5	30.2	41.2	6.56	62.8
27		2 days	73	65.0	22.8	35.1	6.12	57.3
28		3 days	67	66.5	23.6	35.5	5.61	63.3
29		5 days	70	65.7	21.3	32.4	5.56	58.3
30		7 days	69	67.8	22.0	32.5	5.42	60.0
31		33 days	72	61.7	27.8	45.1	7.19	62.7
32		42 days	80	66.3	32.5	49.1	7.68	63.9

Dog No. 45 ♂ (Mean Weight, 18.6 Kilograms)

Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in plasma, g. per 100 cc.	Protein nitrogen in plasma, g. per 100 cc.	Total phosphorus in plasma, mg. per 100 cc.	Lipide phosphorus in plasma, mg. per 100 cc.	Inorganic phosphorus in plasma, mg. per 100 cc.	Diffusible calcium in plasma, mg. per 100 cc.	Non-diffusible calcium in plasma, mg. per 100 cc.	
17.5	4.08	0.032	0.907	23.0	20.9	4.36	.....	.....	1
17.2	5.39	0.030	1.003	25.4	20.9	5.11	8.97	3.80	2
16.5	6.09	0.031	.....	24.9	18.1	5.02	6.64	6.71	3
.....	5.45	.....	.....	.....	.....	.....	.....	.....	4
18.4	4.99	0.033	1.054	23.3	20.3	5.29	7.91	.....	5
16.5	6.00	0.031	1.157	26.6	19.3	4.85	8.95	6.19	6
22.2	4.85	0.027	1.100	25.3	19.6	5.47	8.99	.....	7
19.5	4.50	0.031	1.144	27.6	18.9	6.36	9.57	4.90	8
18.8	4.75	0.032	1.089	28.5	19.8	6.75	8.98	5.85	9
.....	.....	.....	.....	28.1	20.0	6.93	8.70	6.71	10
.....	.....	.....	.....	.....	.....	.....	.....	.....	11
18.5	3.65	0.032	.....	24.3	18.8	5.13	7.57	6.49	12
14.7	5.45	0.031	1.050	24.9	18.7	5.55	8.54	4.05	13
16.6	5.02	0.026	0.922	27.4	20.4	5.22	7.66	6.59	14
14.7	6.89	0.028	0.865	24.7	17.5	5.49	7.68	4.95	15
14.6	5.52	0.031	0.959	24.6	18.5	5.17	7.53	4.77	16
15.2	6.68	0.029	1.035	26.2	18.6	5.98	8.02	4.91	17
12.4	5.58	0.030	1.021	25.2	17.7	5.24	7.49	4.30	18
18.2	5.96	0.030	1.068	23.2	16.8	5.30	.....	.....	19
18.0	9.11	0.023	1.044	25.0	19.5	6.11	7.98	4.65	20
18.4	6.89	0.022	1.048	25.9	19.0	4.95	6.88	4.38	21
16.8	5.58	0.022	1.042	24.2	19.0	5.19	7.22	4.20	22
.....	.....	.....	.....	23.4	18.2	5.84	7.69	4.97	23
.....	.....	.....	.....	.....	.....	.....	.....	.....	24
17.7	5.02	0.020	1.034	21.0	16.1	5.05	7.50	3.56	25
14.0	6.08	0.024	.....	23.6	18.6	5.27	9.29	.....	26
12.6	7.34	0.022	0.945	19.0	15.0	4.65	8.14	3.19	27
13.6	6.86	0.022	0.959	20.6	16.8	5.46	8.31	3.21	28
11.6	6.55	0.023	0.959	21.2	15.3	4.95	.....	.....	29
10.9	7.16	0.025	0.958	23.1	16.0	5.28	8.37	4.58	30
16.7	11.41	0.025	1.080	23.7	18.3	4.87	6.96	6.93	31
17.9	6.48	0.030	1.069	23.5	17.2	6.03	6.48	6.93	32

Table III. Physical and Chemical Measurements on

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns
1	Pre-hemorrhage	15 days	72	76.2	36.6	48.1	6.64	72.4
2		8 days	76	80.8	39.7	49.2	6.59	74.7
3		4 days	72	84.2	39.0	46.3	6.14	75.4
4		3 days	74	81.3	34.2	42.1	5.52	76.3
5		same day	14	.....	.....	.....	.....	.....
6	First Hemorrhage		278	.....	.....	.....	.....	.....
7	Post-hemorrhage	30 min.	60	74.0	31.8	43.0	5.53	77.8
8		1 day	70	77.1	24.8	32.1	5.00	64.2
9		2 days	77	72.6	22.4	31.0	4.84	64.0
10		3 days	76	76.4	22.2	29.1	4.61	63.1
11		6 days	69	71.0	21.8	30.7	4.72	65.0
12		7 days	70	71.4	19.8	27.7	4.48	61.9
13		13 days	70	71.2	24.2	34.1	5.62	60.7
14	Pre-hemorrhage	17 days	74	76.2	33.3	43.7	6.76	64.6
15		16 days	73	77.3	33.8	43.8	7.18	61.0
16		7 days	72	79.9	33.1	41.4	6.74	61.4
17		6 days	100	80.8	32.2	39.9	6.34	63.0
18		same day	25	.....	.....	.....	.....	.....
19	Second Hemorrhage		386	.....	.....	.....	.....	.....
20	Post-hemorrhage	30 min.	64	68.9	29.5	37.8	6.24	60.5
21		1 day	80	65.3	20.0	30.7	4.84	63.4
22		2 days	75	69.7	20.8	29.9	5.38	55.6
23		3 days	72	68.6	17.9	26.1	4.69	55.6
24		4 days	74	66.7	15.3	23.0	3.90	59.0
25		5 days	74	65.7	16.6	25.2	4.66	54.1
26		29 days	84	72.8	24.4	33.6	5.78	58.2
27		30 days	76	74.3	23.0	31.0	5.90	52.6

Dog No. 46 ♂ (Mean Weight, 17.7 Kilograms)

Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in plasma, g. per 100 cc.	Protein nitrogen in plasma, g. per 100 cc.	Total phosphorus in plasma, mg. per 100 cc.	Lipide phosphorus in plasma, mg. per 100 cc.	Inorganic phosphorus in plasma, mg. per 100 cc.	Diffusible calcium in plasma, mg. per 100 cc.	Non-diffusible calcium in plasma, mg. per 100 cc.	
19.7	11.35	0.032	1.268	22.1	12.8	6.65	8.54	5.78	1
19.5	7.89	0.032	1.254	21.4	12.9	6.91	7.71	5.19	2
17.8	9.71	0.031	1.188	20.6	12.9	5.53	7.90	.....	3
17.3	9.44	0.033	1.140	21.2	11.6	5.37	7.75	5.40	4
.....	.....	.....	.....	20.8	13.6	5.93	7.87	4.37	5
.....	.....	.....	.....	.....	.....	.....	.....	.....	6
16.0	14.04	0.028	1.104	19.0	13.2	4.71	7.82	3.49	7
13.4	9.54	0.024	1.064	20.3	13.0	4.46	7.80	4.68	8
13.0	9.39	0.024	1.072	20.2	12.6	4.99	7.93	4.58	9
12.5	8.34	0.027	1.038	20.4	14.3	5.08	8.19	4.28	10
12.3	7.78	0.025	1.007	19.9	13.2	4.96	8.45	4.21	11
11.5	7.71	0.028	1.025	19.5	12.5	5.00	8.35	4.49	12
13.4	6.98	0.027	1.027	18.8	11.9	5.24	8.71	4.62	13
15.4	8.14	0.029	1.015	18.4	12.6	5.15	7.05	4.34	14
19.1	9.31	0.027	1.117	19.1	13.1	5.24	.....	.....	15
15.0	7.78	0.026	1.075	18.4	11.9	5.85	7.17	4.18	16
15.0	6.78	0.026	1.047	18.6	12.1	5.67	7.30	3.95	17
.....	.....	.....	.....	17.0	9.7	4.51	8.45	3.54	18
.....	.....	.....	.....	.....	.....	.....	.....	.....	19
13.6	6.06	0.027	1.005	16.7	10.6	5.10	8.58	4.47	20
11.3	8.72	0.022	1.005	18.1	10.6	4.49	6.66	4.09	21
13.1	9.59	0.025	0.982	17.4	12.6	4.62	6.82	3.92	22
10.6	7.59	0.028	1.032	19.4	12.6	4.86	6.25	3.81	23
9.1	7.79	0.024	0.970	16.6	10.2	4.21	6.69	4.48	24
10.9	7.05	0.024	1.027	18.4	13.3	4.68	7.06	4.57	25
12.1	8.49	0.023	1.112	16.4	9.4	5.55	5.86	5.50	26
10.2	8.14	0.026	1.111	17.4	.....	5.34	6.29	6.08	27

Table IV. Physical and Chemical Measurements on

		Pre- or post hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns
1	Pre-hemorrhage	12 days	68	83.0	43.3	52.3	6.86	76.2
2		10 days	68	84.9	44.8	52.7	6.79	77.6
3		6 days	70	77.3	38.0	49.1	6.70	73.3
4		5 days	63	77.4	36.5	47.2	6.60	71.5
5		3 days	68	73.4	34.4	46.8	6.80	68.9
6	First Hemorrhage		248	.....	.....	.....	.....	.....
7	Post-hemorrhage	30 min.	68	66.7	26.2	39.3	6.23	63.1
8		1 day	69	66.4	23.9	36.1	5.15	70.1
9		2 days	66	56.8	19.2	33.8	5.12	66.0
10		3 days	66	55.9	18.3	32.8	5.32	61.6
11		4 days	69	.....	.....	29.1	5.32	54.6
12		7 days	63	67.5	23.0	34.0	5.12	66.4
13		9 days	65	66.9	16.7	32.9	5.62	58.5
14	Pre-hemorrhage	21 days	69	77.7	34.9	44.9	7.06	63.6
15		19 days	69	78.5	35.1	44.7	7.29	61.3
16		17 days	65	70.8	28.9	40.8	7.06	57.8
17		14 days	68	76.1	30.6	40.2	6.99	57.5
18		12 days	64	.....	.....	.....	6.60	.....
19		11 days	64	68.6	27.1	39.6	6.44	61.5
20		7 days	24	.....	.....	.....	.....	.....
21		3 days	63	69.4	25.5	36.7	6.54	56.1
22		2 days	45	74.3	28.4	38.3	.....	.....
23		same day	10	.....	.....	.....	.....	.....
24	Second Hemorrhage		306	.....	.....	.....	.....	.....
25	Post-hemorrhage	30 min.	55	65.2	21.9	33.6	5.24	64.1
26		1 day	69	63.4	17.9	28.3	4.96	57.0
27		2 days	66	67.2	18.7	27.8	5.32	52.2
28		4 days	62	67.4	17.7	26.3	5.18	50.8
29		5 days	10	.....	.....	.....	.....	.....
30		7 days	64	68.1	18.3	26.9	4.90	55.0
31		11 days	70	74.4	23.9	26.9	4.96	54.2
32	Pre-hemorrhage	5 days	81	72.0	28.4	39.5	8.10	48.7
33		4 days	69	70.4	25.2	35.9	8.60	41.7
34		3 days	63	69.8	24.6	35.3	8.23	42.9
35		2 days	73	71.1	24.0	33.7	7.17	47.0
36		same day	12	.....	.....	.....	.....	.....
37	Third Hemorrhage		303	.....	.....	.....	.....	.....
38	Post-hemorrhage	30 min.	60	55.7	16.5	29.6	6.01	49.2
39		1 day	65	58.2	14.8	25.4	5.81	43.7
40		2 days	64	60.5	14.5	24.0	5.47	43.9
41		3 days	52	58.7	15.3	26.0	5.14	50.7
42		5 days	62	57.6.	12.7	22.1	4.86	45.5
43	Pre-hemorrhage	6 days	75	69.6	28.2	40.6	7.76	52.4
44		5 days	74	69.9	27.1	38.8	7.61	51.0
45		4 days	74	69.9	25.1	35.9	7.44	48.2
46		3 days	74	67.8	21.8	32.2	7.06	45.6
47		same day	13	.....	.....	.....	.....	.....
48	Fourth Hemorrhage		241	.....	.....	.....	.....	.....
49	Post-hemorrhage	30 min.	60	57.8	17.2	29.7	6.08	48.8
50		1 day	88	70.1	19.0	27.1	5.44	49.8
51		2 days	74	62.6	15.6	24.5	5.69	43.1
52		3 days	72	66.5	16.0	24.1	4.88	49.4
53		4 days	83	65.6	15.4	23.5	4.30	54.6
54		5 days	72	66.5	14.0	21.1	4.86	43.4

Dog No. 47 ♂ (Mean Weight, 18.9 Kilograms)

Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in plasma, g. per 100 cc.	Protein nitrogen in plasma, g. per 100 cc.	Total phosphorus in plasma, mg. per 100 cc.	Lipide phosphorus in plasma, mg. per 100 cc.	Inorganic phosphorus in plasma, mg. per 100 cc.	Diffusible calcium in plasma, mg. per 100 cc.	Non-diffusible calcium in plasma, mg. per 100 cc.	
17.3	7.61	0.037	1.114	22.2	16.3	4.41	.....	.....	1
19.1	5.98	0.038	1.039	22.4	16.8	3.91	6.69	6.14	2
15.0	9.01	0.035	1.046	21.0	17.0	4.11	6.67	4.20	3
14.6	7.04	0.036	0.895	21.7	17.2	4.41	.....	.....	4
14.0	6.34	0.034	1.062	20.5	17.3	4.04	5.93	5.39	5
.....	.....	.....	.....	.....	.....	.....	.....	.....	6
11.8	7.46	0.032	0.819	17.7	14.4	3.72	6.31	5.00	7
12.3	8.86	0.033	0.941	21.4	18.4	4.25	6.53	6.66	8
13.4	7.70	0.029	0.944	20.6	16.9	4.15	6.78	4.95	9
12.6	8.21	0.026	0.924	26.4	20.2	3.64	5.87	4.02	10
11.2	7.89	0.024	0.874	22.0	18.5	4.34	6.55	4.26	11
14.0	9.05	0.030	0.975	22.9	17.2	4.61	6.23	4.86	12
13.6	9.00	0.031	0.962	23.7	17.1	4.74	6.43	3.03	13
.....	8.36	0.034	1.041	21.5	18.2	4.37	7.26	3.54	14
15.8	8.85	0.032	0.938	23.2	18.5	3.83	.....	.....	15
15.2	9.18	0.032	1.030	25.8	17.6	4.64	8.48	3.49	16
15.2	8.66	0.033	0.946	19.9	17.9	3.86	6.29	6.47	17
.....	11.15	.....	.....	.....	.....	.....	.....	.....	18
12.4	9.43	0.033	0.992	24.3	18.8	4.70	7.44	3.00	19
.....	.....	.....	.....	28.6	20.6	4.38	7.56	4.20	20
13.3	8.88	0.034	1.066	24.9	18.4	5.31	8.99	3.50	21
.....	.....	.....	.....	25.3	20.1	4.79	10.67	4.12	22
.....	.....	.....	.....	24.4	18.1	4.72	9.47	4.76	23
.....	.....	.....	.....	.....	.....	.....	.....	.....	24
13.4	8.62	0.033	1.019	21.5	16.4	4.49	7.72	5.81	25
11.0	10.34	0.029	0.953	22.8	18.7	4.10	7.64	6.74	26
11.3	12.48	0.028	0.971	25.1	18.5	4.80	8.04	5.16	27
11.1	10.99	0.028	1.042	23.7	.....	.....	7.30	.....	28
.....	.....	.....	.....	26.1	19.6	5.54	8.29	4.53	29
6.5	10.44	0.030	1.024	25.1	19.2	4.79	8.99	4.32	30
9.3	12.01	0.026	1.041	26.6	17.4	5.48	7.89	4.64	31
10.1	6.20	0.028	1.108	25.9	22.0	4.58	7.55	6.37	32
14.6	12.94	0.027	1.112	25.9	19.0	5.47	8.46	4.22	33
13.6	8.91	0.029	1.055	29.6	19.2	6.82	7.66	3.80	34
13.0	10.06	0.027	1.072	25.8	21.3	5.01	7.37	4.27	35
.....	.....	.....	.....	26.2	21.2	5.06	7.80	4.49	36
.....	.....	.....	.....	.....	.....	.....	.....	.....	37
11.5	7.38	0.029	0.974	23.5	18.8	4.20	7.83	4.01	38
10.2	11.02	0.027	0.922	26.1	20.4	4.98	7.98	5.76	39
10.0	10.02	0.024	1.026	25.7	19.4	4.96	7.96	4.33	40
8.0	9.38	0.027	1.015	24.8	19.7	4.88	7.39	5.20	41
8.1	17.82	0.028	1.018	27.3	19.5	5.20	7.76	4.44	42
13.5	6.68	0.025	1.088	23.5	18.6	5.29	7.28	5.40	43
13.1	7.86	0.024	1.108	27.4	16.5	5.14	7.50	4.30	44
13.2	7.56	0.024	1.103	26.7	22.3	4.53	6.30	3.56	45
12.4	7.10	0.022	1.046	25.3	21.5	4.49	5.76	6.82	46
.....	.....	.....	.....	26.4	20.0	5.79	6.08	5.55	47
.....	.....	.....	.....	.....	.....	.....	.....	.....	48
12.3	7.32	0.024	.....	22.9	16.7	5.00	5.78	4.57	49
9.6	8.10	0.024	0.978	24.6	19.2	4.77	6.87	5.06	50
10.1	9.09	0.021	1.015	23.4	19.1	4.82	6.95	5.84	51
9.1	8.40	0.023	1.001	23.4	18.6	4.90	5.74	4.11	52
8.3	8.24	0.021	1.000	23.1	18.2	4.12	7.49	4.62	53
8.2	9.26	0.020	0.961	22.1	14.6	4.50	5.92	5.21	54

Table V. Physical and Chemical Measurements on Dog No. 20 ♀ (Mean Weight, 19.3 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Nonprotein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	
1	Pre-hemorrhage	56 days	22	62.2	23.6	38.0	5.65	67.3	.....	3.90	.....	.....	1
2		14 days	31	65.8	30.4	46.2	.....	.....	.....	.....	0.030	2.896	2
3		7 days	48	70.1	31.7	45.2	.....	.....	12.4	.....	0.030	2.961	3
4		3 days	40	68.6	27.2	39.7	6.98	56.9	14.2	9.55	0.028	2.863	4
5		same day	45	59.5	22.8	38.3	6.08	63.0	12.8	9.70	0.030	2.424	5
6	First Hemorrhage		263	.....	.....	.....	.....	.....	.....	.....	.....	.....	6
7		same day	45	71.2	22.8	32.1	5.97	53.8	13.8	7.05	0.029	2.295	7
8	Second Hemorrhage		228	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
9	Post-hemorrhage	2 days	34	59.8	19.3	32.2	5.64	57.1	9.8	5.40	0.034	2.200	9
10		1 week	39	64.2	19.7	35.1	7.51	46.7	12.7	8.95	0.032	2.528	10
11		2 weeks	73	56.1	14.6	26.7	4.98	53.6	11.5	7.10	0.035	2.240	11
12		3 weeks	40	64.6	20.4	30.7	5.85	52.5	9.1	6.55	0.028	2.011	12
13		4 weeks	42	59.5	19.8	33.3	7.63	43.6	9.5	7.20	0.031	2.359	13
14		5 weeks	44	59.8	17.8	30.1	5.62	53.6	7.1	9.80	0.028	2.242	14
15		6 weeks	44	64.0	18.9	29.5	5.66	52.1	10.7	5.30	0.029	2.080	15
16		7 weeks	38	60.7	20.2	33.3	6.28	53.0	8.5	5.35	0.034	2.346	16
17		8 weeks	44	69.3	26.8	38.6	6.12	63.1	8.5	5.00	0.029	2.110	17
18		9 weeks	49	61.0	21.7	35.5	6.41	55.4	9.4	6.95	0.033	2.352	18
19		10 weeks	48	63.8	21.7	34.1	8.12	42.0	10.6	6.25	0.037	2.368	19
20		11 weeks	47	64.8	19.3	29.7	7.00	42.4	8.0	9.05	0.028	2.582	20
21		12 weeks	46	65.9	21.5	32.6	7.02	46.4	10.7	6.10	0.032	2.613	21

Table VI. Physical and Chemical Measurements on Dog No. 21 ♀ (Mean Weight, 11.7 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Nonprotein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	
1	Pre-hemorrhage	39 days	30	78.9	37.2	47.1	.....	.....	.....	.....	0.027	3.106	1
2		30 days	42	70.7	30.7	43.4	.....	.....	12.9	.....	0.028	3,098	2
3		28 days	42	63.6	26.3	41.3	6.28	65.8	13.1	6.50	0.029	3.047	3
4		11 days	41	68.5	30.9	44.8	7.08	63.3	14.7	3.25	0.026	3.140	4
5		same day	41	56.4	27.0	48.0	7.73	62.1	14.7	18.35	0.038	3.067	5
6	First Hemorrhage		154	.....	.....	.....	.....	.....	.....	.....	.....	.....	6
7		same day	43	58.6	22.3	38.0	6.35	59.8	12.8	17.15	0.030	2.738	7
8	Second Hemorrhage		152	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
9	Post-hemorrhage	2 days	41	69.4	22.1	31.9	4.80	66.4	11.6	7.00	0.035	2.480	9
10		1 week	45	63.8	25.2	39.6	7.10	55.8	13.5	13.45	0.033	2.763	10
11		2 weeks	45	66.3	24.0	36.2	6.64	54.5	11.3	7.15	0.028	2.457	11
12		3 weeks	42	55.4	23.1	42.6	7.36	57.9	12.2	4.75	0.037	2.658	12
13		4 weeks	44	62.2	26.8	43.0	8.15	52.8	12.0	5.40	0.030	2.741	13
14		5 weeks	44	60.9	23.9	39.3	7.44	52.8	11.7	5.15	0.026	2.604	14
15		6 weeks	44	63.4	25.4	40.1	8.67	46.2	10.8	10.25	0.032	2.588	15
16		7 weeks	47	66.6	24.8	37.5	7.90	47.5	11.2	5.60	0.025	2.445	16
17		8 weeks	46	63.9	24.4	38.2	.....	.....	12.4	9.80	0.033	2.798	17
18		9 weeks	42	56.7	22.4	39.5	8.23	48.0	.....	.....	.....	.....	18

Table VII. Physical and Chemical Measurements on Dog No. 22 ♀ (Mean Weight, 12.9 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Nonprotein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	
1	Pre-hemorrhage	12 days	41	61.8	28.1	45.4	6.18	73.5	18.5	4.15	0.039	2.812	1
2		same day	41	51.5	23.1	44.9	7.35	61.1	16.2	10.45	0.035	2.924	2
3	First Hemorrhage		159	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
4		same day	43	55.0	18.8	34.2	5.54	61.7	12.7	9.40	0.035	2.420	4
5	Second Hemorrhage		163	.....	.....	.....	.....	.....	.....	.....	.....	.....	5
6	Post-hemorrhage	3 days	42	59.4	20.8	34.9	4.54	76.9	10.4	10.20	0.053	2.272	6
7		1 week	38	50.7	17.6	34.7	4.89	71.0	9.2	15.60	0.052	2.297	7
8		2 weeks	47	58.3	22.3	38.2	6.75	56.6	9.9	13.45	0.040	2.445	8
9		3 weeks	47	58.6	21.9	37.3	6.01	62.1	8.8	9.25	0.035	2.525	9
10		4 weeks	45	57.1	22.8	40.0	6.76	59.2	10.7	7.30	0.038	2.562	10
11		5 weeks	46	61.0	26.9	44.1	7.54	58.5	11.6	8.80	0.047	2.694	11
12		6 weeks	45	60.7	28.7	47.3	7.02	67.4	11.0	4.50	0.042	2.668	12
13		7 weeks	45	59.3	29.6	50.0	7.89	63.4	12.8	6.05	0.049	2.859	13
14		8 weeks	46	60.3	26.0	43.2	7.27	59.4	11.9	6.60	0.047	2.724	14
15		9 weeks	47	61.9	28.2	45.6	7.78	58.6	14.6	8.45	0.040	2.811	15

Table VIII. Physical and Chemical Measurements on Dog No. 19 ♀ (Mean Weight, 18.0 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	Amino acid nitrogen in whole blood, mg. per 100 cc.	Creatinine in whole blood, mg. per 100 cc.	
1	Pre-hemorrhage	30 days	50	67.7	39.6	58.4	7.54	6.80	77.4	24.5	3.60	0.031	3.479	12.73	.....	1.72	1
2		27 days	51	.....	.....	59.8	7.00	6.90	85.4	22.8	4.55	0.037	3.445	18.02	.....	1.72	2
3		3 days	20	70.2	41.3	58.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
4		same day	49	.....	.....	.....	.....	.....	.....	21.6	.....	0.036	3.284	19.30	4.05	1.65	4
5	First Hemorrhage		238	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5
6		same day	50	.....	.....	.....	.....	.....	.....	17.8	.....	0.037	2.833	22.48	15.32	1.66	6
7	Second Hemorrhage		247	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7
8	Post-hemorrhage	2 days	49	.....	.....	.....	5.06	6.88	.....	16.0	4.15	0.033	2.617	18.61	16.73	1.65	8
9		4 days	50	64.4	22.7	35.2	5.10	6.91	69.0	14.3	5.85	0.028	2.492	16.37	15.60	1.57	9
10		1 week	52	56.1	22.1	39.3	4.98	6.84	78.9	16.4	5.55	0.036	2.774	25.07	17.27	1.63	10
11		2 weeks	49	56.3	22.0	39.1	5.74	6.51	68.1	14.5	4.50	0.033	2.637	20.39	18.10	1.66	11
12		3 weeks	51	.....	.....	.....	5.66	6.43	.....	15.0	5.15	0.031	2.469	14.83	21.12	1.58	12
13		4 weeks	52	59.7	21.8	36.6	6.11	6.12	59.9	15.2	4.45	0.038	2.672	18.80	16.25	1.55	13
14		5 weeks	51	52.6	19.6	37.2	6.38	6.21	58.3	13.8	3.60	0.031	2.649	16.94	17.11	1.56	14
15		6 weeks	52	50.8	18.6	36.7	5.88	6.24	62.4	12.2	3.90	0.035	2.305	18.80	7.08	1.38	15
16		7 weeks	52	53.2	18.6	34.9	6.68	6.27	52.2	13.0	3.90	0.031	2.499	14.99	16.40	1.50	16
17		8 weeks	53	52.5	18.9	36.1	7.38	6.10	48.9	11.8	2.60	0.024	2.186	10.75	6.50	1.49	17
18		9 weeks	51	51.3	19.3	37.6	7.48	6.13	50.3	13.7	2.60	0.034	2.406	13.55	14.12	1.49	18
19		10 weeks	50	48.9	17.9	36.7	7.62	5.95	48.2	12.2	3.50	0.025	2.345	3.46	9.98	1.52	19
20		11 weeks	50	48.1	16.6	34.5	8.32	5.94	41.5	10.6	3.05	0.030	2,340	17.05	8.90	1.51	20
21		12 weeks	51	47.1	16.5	35.1	8.08	5.87	43.4	10.8	3.35	0.029	2.276	13.21	16.65	1.52	21

Table IX. Physical and Chemical Measurements on Dog No. 32 ♀ (Mean Weight, 17.6 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	
1	Pre-hemorrhage	23 days	43	.....	.....	.....	6.74	7.03	.....	.....	9.05	0.035	3.420	.....	1
2		18 days	49	68.8	32.9	47.9	6.63	7.13	72.3	15.4	9.45	0.042	2.974	.....	2
3		16 days	49	.....	.....	.....	6.81	7.40	.....	19.7	8.35	0.046	3.437	7.98	3
4		14 days	48	61.6	28.6	46.9	6.50	7.25	72.2	21.0	7.95	0.042	3.171	6.84	4
5		7 days	58	69.0	34.2	49.6	7.21	6.93	68.8	19.7	7.75	0.032	3.135	7.40	5
6		same day	58	.....	.....	.....	.....	.....	.....	14.1	.....	0.037	2.995	14.25	6
7	First Hemorrhage		183	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7
8		same day	42	.....	.....	.....	.....	.....	.....	14.8	.....	0.038	2.899	22.43	8
9	Second Hemorrhage		200	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	9
10	Post-hemorrhage	1 day	49	56.6	19.1	33.8	4.66	6.77	72.5	11.8	8.40	0.036	2.470	17.89	10
11		3 days	66	58.4	19.4	33.3	4.80	6.69	69.3	11.1	11.80	0.033	2.573	17.76	11
12		5 days	50	57.6	19.2	33.8	4.73	6.86	71.4	11.3	10.15	0.032	2.472	15.35	12
13		1 week	55	60.1	18.7	31.1	4.76	6.70	65.3	11.1	7.95	0.031	2.445	9.75	13
14		2 weeks	47	57.6	19.5	33.8	5.09	6.26	67.5	11.0	7.70	0.033	2.323	19.61	14
15		3 weeks	50	57.1	19.0	33.6	4.82	6.37	69.7	11.1	9.00	0.032	2.411	22.46	15
16		4 weeks	62	59.0	21.3	36.2	5.64	6.49	64.2	11.9	6.95	0.034	2.444	.....	16
17		5 weeks	51	57.1	19.9	34.9	5.20	6.44	67.1	12.3	7.45	0.030	2.520	16.86	17
18		6 weeks	51	56.4	20.4	36.1	5.99	6.28	60.3	12.3	8.90	0.034	2.567	11.27	18
19		7 weeks	41	58.3	20.3	34.9	5.80	6.53	60.2	13.1	11.10	.....	.....	.....	19
20		9 weeks	35	60.0	21.7	36.3	5.78	6.54	62.8	13.4	7.10	.....	.....	.....	20
21		11 weeks	54	58.9	22.4	38.1	6.19	6.45	61.6	13.9	6.75	.....	.....	.....	21
22		12 weeks	38	57.5	21.5	37.5	5.98	6.50	62.7	13.8	6.75	.....	.....	.....	22

Table X. Physical and Chemical Measurements on Dog No. 17 ♂ (Mean Weight, 16.8 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	Amino acid nitrogen in whole blood, mg. per 100 cc.	Creatinine in whole blood, mg. per 100 cc.	
1	Pre-hemorrhage	94 days	52	74.9	38.5	51.4	7.54	7.30	68.2	17.1	8.05	.....	.....	6.78	.....	1.41	1
2		90 days	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.92	.....	1.43	2
3		80 days	10	.....	.....	.....	.....	.....	.....	.....	.....	0.028	3.449	.....	.....	.....	3
4		32 days	53	69.8	32.7	46.8	7.89	6.90	59.3	18.9	5.30	0.030	3.331	13.74	.....	1.44	4
5		same day	50	.....	.....	.....	.....	.....	.....	19.8	.....	0.028	3.277	12.26	4.84	1.59	5
6	First Hemorrhage		240	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6
7		same day	50	.....	.....	.....	.....	.....	.....	18.6	.....	0.030	3.205	18.48	5.69	1.66	7
8	Second Hemorrhage		225	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
9	Post-hemorrhage	2 days	80	.....	.....	.....	5.73	6.77	.....	13.9	9.45	0.027	2.523	14.23	7.34	1.57	9
10		5 days	51	59.1	24.4	41.2	6.11	6.50	67.4	16.1	8.05	0.031	2.729	15.83	15.52	1.56	10
11		6 days	50	63.7	22.3	35.0	6.14	6.60	57.0	14.4	7.80	0.027	2.748	12.92	6.82	1.54	11
12		1 week	49	64.0	22.6	35.4	6.15	6.68	57.6	13.5	9.45	0.024	2.441	11.00	14.62	1.61	12
13		2 weeks	50	64.6	22.9	35.5	7.10	6.26	50.0	14.6	9.55	0.027	2.843	13.71	15.92	1.58	13
14		3 weeks	49	69.1	29.0	42.1	6.97	6.40	60.4	13.5	10.10	0.028	2.657	14.72	12.27	1.58	14
15		4 weeks	44	61.7	23.9	38.7	7.88	6.07	49.1	15.1	5.45	0.026	2.954	9.11	7.52	1.55	15
16		5 weeks	44	58.5	22.8	39.0	7.44	6.22	52.4	14.7	7.15	0.027	2.743	11.52	9.73	1.58	16
17		6 weeks	60	65.4	24.4	37.4	8.04	6.18	46.5	14.0	12.00	0.026	2.764	10.86	12.97	1.56	17
18		7 weeks	51	63.0	22.0	35.0	7.44	6.25	47.0	11.8	6.85	0.023	2.487	12.08	4.47	1.50	18
19		8 weeks	52	65.3	22.2	34.0	7.94	6.08	42.8	12.1	7.30	0.026	2.409	11.19	3.06	1.50	19
20		9 weeks	52	63.0	21.6	34.2	7.12	6.18	48.0	11.7	8.30	0.030	2.740	10.08	4.50	1.50	20
21		10 weeks	53	67.1	23.6	35.1	8.03	6.05	43.7	11.7	8.10	0.026	2.474	5.98	12.67	1.49	21
22		11 weeks	50	70.6	24.6	34.9	7.88	6.02	44.3	10.7	7.10	0.025	2.545	3.40	10.63	1.50	22
23		12 weeks	62	.....	.....	.....	7.46	5.86	46.9	11.3	9.30	0.029	2.716	11.47	8.19	1.52	23

Table XI. Physical and Chemical Measurements on Dog No. 18 ♂ (Mean Weight, 20.8 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	Amino acid nitrogen in whole blood, mg. per 100 cc.	Creatinine in whole blood, mg. per 100 cc.	
1	Pre-hemorrhage	84 days	53	53.4	23.1	43.2	6.20	7.00	69.7	.....	3.15	0.026	2.875	.....	.....	1.42	1
2		61 days	15	.....	.....	.....	.....	.....	.....	.....	.....	0.025	2.831	8.79	.....	1.48	2
3		54 days	15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.80	.....	1.43	3
4		33 days	15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.74	.....	1.49	4
5		10 days	50	53.4	25.8	48.4	7.07	6.90	68.5	17.4	6.75	0.034	3.012	13.55	.....	1.64	5
6		7 days	52	58.4	26.9	46.1	6.99	6.80	66.0	17.3	5.10	0.030	3.387	13.79	.....	1.81	6
7		same day	51	.....	.....	.....	.....	.....	.....	17.4	.....	0.031	3.431	12.32	.....	1.76	7
8	First Hemorrhage		210	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
9		same day	50	.....	.....	.....	.....	.....	.....	14.1	.....	0.031	2.454	19.64	.....	1.64	9
10	Second Hemorrhage		241	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	10
11	Post-hemorrhage	1 day	51	54.9	16.8	30.6	5.24	6.57	58.4	12.7	7.55	0.028	2.412	15.36	.....	1.65	11
12		3 days	45	49.5	16.4	33.0	4.67	6.61	70.7	9.8	6.65	0.030	2.195	19.15	4.94	1.68	12
13		5 days	50	50.7	17.4	34.2	4.48	6.35	76.3	.....	8.80	0.029	2.286	13.89	.....	1.69	13
14		1 week	45	50.0	16.6	33.3	4.54	6.50	73.4	.....	6.80	0.030	2.285	17.17	3.61	1.74	14
15		2 weeks	48	48.5	17.8	35.5	6.34	6.28	56.0	10.4	7.20	0.029	2.236	17.35	8.74	1.63	15
16		3 weeks	50	56.8	19.2	33.8	6.20	6.22	54.5	11.0	8.70	0.029	2.336	14.83	10.56	1.70	16
17		4 weeks	49	60.3	22.1	36.6	6.48	6.37	56.5	10.5	5.80	0.027	2.298	12.82	12.86	1.70	17
18		5 weeks	72	54.2	19.3	35.7	6.48	6.21	55.1	11.6	6.50	0.029	2.396	14.21	13.11	1.66	18
19		6 weeks	50	53.2	18.1	34.1	6.58	6.20	51.8	11.4	4.25	0.028	2.262	12.77	20.98	1.70	19
20		7 weeks	50	59.3	21.0	35.3	6.45	6.02	54.7	11.4	7.40	0.028	2.337	10.44	18.93	1.67	20
21		8 weeks	49	57.4	21.5	37.5	7.10	5.87	52.8	11.2	4.65	0.030	2.545	9.71	9.03	1.72	21
22		9 weeks	37	.....	.....	.....	7.01	6.08	.....	10.8	3.90	0.030	2.285	17.27	8.00	1.63	22
23		10 weeks	57	53.6	19.2	35.9	7.85	5.94	45.7	11.5	4.90	0.027	2.358	12.53	6.73	1.65	23
24		11 weeks	54	57.9	18.6	32.1	7.30	5.96	44.0	11.2	3.75	0.028	2.182	10.65	3.51	1.67	24
25		12 weeks	53	56.1	20.1	35.8	7.68	5.87	46.6	10.5	5.40	0.024	2.341	6.89	10.42	1.70	25

Table XII. Physical and Chemical Measurements on Dog No. 30 ♂ (Mean Weight, 17.0 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	
1	Pre-hemorrhage	14 days	50	.....	.....	.....	7.19	7.03	.....	.....	10.40	0.052	3.191	.....	1
2		12 days	41	.....	.....	.....	7.08	6.73	.....	.....	11.35	0.047	3.303	9.18	2
3		7 days	49	75.9	32.3	42.6	6.31	7.04	67.5	.....	9.95	0.046	3.338	6.15	3
4		5 days	51	75.6	29.7	39.3	6.00	7.06	65.5	17.6	9.65	0.041	2.697	7.28	4
5		2 days	49	75.4	29.4	39.0	6.14	7.03	63.5	17.3	8.80	0.035	2.938	8.88	5
6		same day	50	.....	.....	.....	.....	.....	.....	17.2	.....	0.034	2.674	7.46	6
7	First Hemorrhage		220	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7
8		same day	50	.....	.....	.....	.....	.....	.....	15.4	.....	0.027	2.584	6.40	8
9	Second Hemorrhage		215	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	9
10	Post-hemorrhage	1 day	51	72.0	22.5	31.2	5.35	7.03	58.3	10.5	8.75	0.032	2.355	6.82	10
11		3 days	50	74.4	22.8	30.6	5.19	6.84	58.9	12.0	6.80	0.035	2.505	6.86	11
12		5 days	51	72.6	25.1	34.6	4.86	6.76	71.2	11.6	7.15	0.036	2.473	6.89	12
13		1 week	89	73.3	24.3	33.2	5.31	6.72	.....	13.0	5.25	0.039	2.118	17.78	13
14		2 weeks	49	78.1	29.3	37.6	5.99	6.77	62.8	13.2	17.00	0.038	2.224	25.53	14
15		3 weeks	49	78.8	31.6	40.0	5.93	6.70	67.4	12.9	8.80	0.036	2.820	21.33	15
16		4 weeks	50	75.4	32.1	42.6	6.50	6.60	65.5	15.6	5.70	0.034	2.944	21.55	16
17		5 weeks	49	85.6	38.8	45.4	7.08	6.56	64.1	15.7	7.85	0.033	3.053	21.88	17
18		6 weeks	50	79.9	33.5	43.0	6.62	6.55	64.9	14.8	6.50	0.032	2.788	.....	18
19		8 weeks	49	85.2	38.4	45.1	7.32	6.59	61.6	16.2	6.55	0.034	2.939	13.64	19
20		10 weeks	35	77.4	34.6	44.7	6.30	6.66	70.9	17.9	5.15	.....	.....	.....	20
21		12 weeks	38	74.8	34.6	46.3	7.40	6.51	62.5	16.9	6.55	.....	.....	.....	21

Table XIII. Physical and Chemical Measurements on Dog No. 31 ♂ (Mean Weight, 21.1 Kilograms)

		Pre- or post-hemorrhagic time interval	Blood removed, in cc.	Total blood volume, in cc. per kg.	Cell volume, in cc. per kg.	Cell volume per cent	Red cell count, in millions	Red cell diameter, in microns	Mean corpuscular volume, in cubic microns	Oxygen-combining capacity, in volumes per cent	White cell count, in thousands	Non-protein nitrogen in whole blood, g. per 100 cc.	Protein nitrogen in whole blood, g. per 100 cc.	Urea nitrogen in whole blood, mg. per 100 cc.	
1	Pre-hemorrhage	17 days	50	81.5	39.4	48.4	6.89	7.08	70.3	16.3	10.00	0.045	3.517	.....	1
2		14 days	49	74.4	36.1	48.6	6.33	7.26	76.8	15.4	10.75	0.044	3.309	8.43	2
3		7 days	49	72.2	32.8	45.5	6.54	7.23	69.6	19.2	8.05	0.043	3.144	7.54	3
4		5 days	50	71.4	30.4	42.6	6.46	7.25	65.9	18.7	10.45	0.035	3.318	7.68	4
5		same day	50	.....	.....	.....	.....	.....	.....	12.5	.....	0.039	2.883	14.76	5
6	First Hemorrhage		282	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6
7		same day	50	.....	.....	.....	.....	.....	.....	11.4	.....	0.033	2.563	7.59	7
8	Second Hemorrhage		282	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
9	Post-hemorrhage	1 day	50	62.0	20.4	32.9	4.45	6.96	73.9	11.9	8.60	0.036	2.583	20.51	9
10		3 days	49	67.1	23.6	35.2	4.59	7.04	76.7	10.5	9.10	0.037	2.934	17.62	10
11		5 days	49	67.9	24.5	36.0	4.85	6.84	74.3	11.3	10.80	0.043	2.696	18.43	11
12		1 week	52	69.3	24.6	35.5	4.10	6.90	.....	11.4	8.25	0.039	2.047	25.02	12
13		2 weeks	50	70.6	27.6	38.9	5.64	6.70	69.0	12.6	8.40	0.038	2.706	16.55	13
14		3 weeks	48	70.6	28.9	40.9	6.34	6.65	64.5	13.4	7.50	0.037	2.804	30.65	14
15		4 weeks	52	72.0	31.7	44.1	6.14	6.67	71.8	14.0	8.65	0.035	2.892	23.99	15
16		5 weeks	51	75.3	34.8	46.3	6.73	6.67	68.8	16.9	8.15	0.036	2.950	.....	16
17		6 weeks	48	71.1	30.7	43.2	6.80	6.81	63.6	15.9	8.10	0.038	2.956	31.91	17
18		8 weeks	51	76.3	32.5	43.2	6.48	6.91	66.6	16.5	7.30	0.038	3.078	11.52	18
19		10 weeks	37	72.6	32.2	44.3	6.53	6.72	67.9	16.3	7.55	.....	.....	.....	19
20		12 weeks	38	70.6	32.5	46.1	6.39	6.59	72.1	15.2	9.00	.....	.....	.....	20